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CARCINOGENESIS BY ULTRAVIOLET RAYS WITH REFERENCE TO WAVELENGTH AND ENERGY

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The value of ultraviolet rays as a carcinogenic agent has been repeatedly demonstrated.[1] The agent is potent, the dose can be readily controlled, and the incidence of tumors produced is high. Furthermore, the tumors appear on the surface, where the preliminary stages can be easily followed. Nevertheless, little information is available regarding the physical factors concerned in the production of cancer with ultraviolet rays. The necessary dosage has not been accurately defined, nor is it known precisely which wavelengths are carcinogenic. Our purpose in the following experiment was to determine these two points.

METHODS

The general experimental procedure consisted in exposing white mice to radiation of known spectral distribution and energy content and noting the number of tumors formed. Six different sources of radiant energy were used, so selected that the relative carcinogenic effectiveness of the various spectral regions could be compared. Two Burdick lamps of the standard clinical type were employed. The rays were emitted by quartz mercury vapor Uviarc lamps about 12 cm. long, mounted under semiparabolic chromium reflectors. Current was supplied from a 110 volt line through special rectifiers. The lamps operated at an average of 70 and 55 volts, respectively, both at an amperage of 8, although at times the voltage fluctuated by as much as 10 volts due to line variation and cooling effects.

As sources of radiant energy, which consisted predominantly of the shorter ultraviolet rays, two quartz mercury vapor glow lamps (cold quartz lamps) were used. One was a small Fluorolight lamp, the essential element of which was a straight

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1. (*a*) Rusch, H. P., and Baumann, C. A.: Am. J. Cancer **35**:55, 1939. (*b*) Roffo, A. H.: Lancet **1**:472, 1936. (*c*) Beard, H. H.; Bogges, T. S., and von Haam, E.: Am. J. Cancer **27**:257, 1936.

quartz tube 12 cm. long, containing mercury vapor at low pressure. Light from the other lamp was emitted by a quartz tube 175 cm. long, shaped into a plane hexagonal spiral and filled with mercury vapor at low pressure. Both lamps were equipped with reflectors, and operated from 110 volt lines through self-contained transformers; 89 per cent of the energy emitted was of wavelength 2,537 angstroms. A small fraction of the radiation was visible, and measurements made with our special 986–$NiSO_4$ filter (see later paragraph) indicated that about 0.5 per cent of the total emission of the smaller lamp and 7 per cent of the larger consisted of wavelengths 2,967 to 3,130 angstroms.

As a source of visible radiation, a 500 watt tungsten Mazda light bulb provided with a metal reflector and operated from the 110 volt circuit was used. The ultraviolet emission of this light was a very small percentage of the total radiation, almost all the energy being in the visible and infra-red regions. Studies on the effectiveness of radiations of shorter wavelengths, 1 to 3 angstroms, were also made. Such energy was produced by a grenz ray generator.

The use of these different lamps by themselves could have given only a very general indication of the carcinogenic potency of the various wavelengths of radiation. Further selection of wavelength bands was therefore made by the use of appropriate filters. These consisted of polished glasses, 6 inches (15 cm.) square and 2 to 4 mm. thick, which were fastened to the Uviarc lamps by means of special holders. The following filters were used: Corning 970, which transmitted 2,900 angstroms and above; Corning 352, which transmitted above 4,700 angstroms, and ordinary window glass, which transmitted above 3,341 angstroms. In addition, a special combination filter was employed, consisting of Corning filter 986 plus an eighty-six hundredths molar aqueous solution of nickel sulfate contained in a special cell made of two polished Corning 791 filters, 2 mm. thick and 14 cm. (5⅝ inches) square and separated by 1.5 cm. The cell was provided with an outlet to which a small leveling bulb was attached with rubber tubing. This was maintained above the level of the filter during use, and served as an overflow when the solution expanded from the heat. The 986 filter was placed just below the $NiSO_4$ cell, and the whole device was supported beneath the lamp by means of a special holder. This combination virtually transmitted only the 2,967, 3,021 and 3,130 angstrom lines of the mercury spectrum.

The quantity of energy emitted by the various lamps was determined in four different ways, each of which measured a different fraction of the energy spectrum. The first measurement was made by means of a thermopile placed at various distances from the lamp, and the data were plotted in the form of a calibration curve (fig. 1). Since the thermopile measures ultraviolet, visible and infra-red energy, the readings were considered to be an index of the "complete" energy spectrum emitted.

A second method of determining energy output was by means of the uranyl oxalate actinometer,[2] in which a solution of twentieth-molar oxalic acid and hundredth-molar uranyl nitrate contained in a deep vessel was exposed to the radiation and the decomposition of the oxalate determined at intervals by titration of aliquots with potassium permanganate. The amount of oxalate decomposed is proportional to the amount of energy applied in the form of rays of wavelengths 2,000 to 4,500 angstroms. The energy delivered by the source could therefore be calculated, assuming an average quantum yield of 0.57 throughout the range measured.[2] The values obtained in this manner are designated "ultraviolet" energy.

The third method of measuring energy made use of the two procedures previously indicated but differed in that the radiation was first passed through the

2. Leighton, W. G., and Forbes, G. S.: J. Am. Chem. Soc. **52**:3139, 1930.

special 986-$NiSO_4$ filter. Since the filter absorbed 60 per cent of the energy in its transmittance bands, it was necessary to multiply the values by 2.5 in order to get a corrected figure of energy output for these bands without the filter. The transmission of mercury lines through this filter was determined, using a large quartz monochromator. Since the bands transmitted by this filter (2,967, 3,021 and 3,130 angstroms) were near the middle range of ultraviolet wavelengths, the energy so measured was designated as "midultraviolet" energy. The energy levels of the three designated regions differed somewhat in the two Burdick Uviarc lamps. Calibration curves of each lamp for the three regions at various heights were determined and plotted (fig. 1). All energy levels are expressed as ergs per square centimeter per second (ergs / sq. cm. / sec.).

The fourth method of measuring the energy output of our lamps made use of a titanium photoelectric cell. These measurements were made by Dr. Harold F.

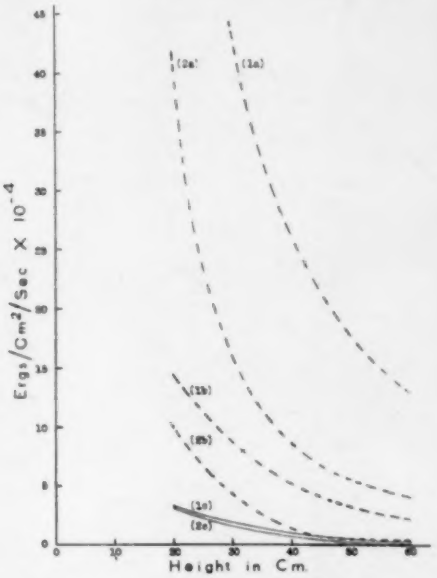

Fig. 1.—Curves showing the relationship between the "complete," "ultraviolet" and "midultraviolet" energy output of Burdick lamps 1 and 2: (1*a*) "complete" energy from lamp 1, (2 *a*) "complete" energy from lamp 2, (1*b*) "ultraviolet" energy from lamp 1, (2 *b*) "ultraviolet" energy from lamp 2, (1 *c*) "midultraviolet" energy from lamp 1, (2 *c*) "midultraviolet" energy from lamp 2.

Blum, of the National Cancer Institute. In table 1 are listed the values for the "midultraviolet" energy from the Burdick lamps and the "ultraviolet" energy emitted from the cold quartz lamps. The titanium cell is particularly sensitive to the short ultraviolet rays, and calculations of energy values for any spectral region depend on measurements of the spectral distribution for any given lamp. Unfortunately, the variations in line voltage in our institute are sufficiently great to alter this spectral distribution from time to time. Considering these facts, the values obtained by the two methods agree remarkably well. For the present discussion, we have used only the values obtained with the first three methods,

because they were available for the calibration of our lamps throughout the experiments and are average values rather than readings obtained in a single period.

Young adult white ABC mice of both sexes were used in all experiments except two in which white mice from a stock colony were used. They were kept in ordinary cages with Purina dog chow [3] and water available at all times except for the short period each day when they were transferred to a special cage for irradiation. This cage was 25.5 cm. (10 inches) square and 3 cm. (1¼ inches) deep. It was constructed entirely of wire mesh and was divided into 24 individual rectangular compartments, each just large enough to hold 1 mouse (fig. 2). Thus previous sources of error due to huddling of the animals or to their movement about the irradiation cage were considerably decreased.[1]

The animals were irradiated on six or seven days of each week. Four irradiation cages were placed under the lamp at one time, except when the filters were used, when only one cage was irradiated at a time. There was a considerable difference in the energy received by the mice directly under the lamp as compared

TABLE 1.—*Comparison of Energy Values Obtainable with the Titanium Photoelectric Cell and the Uranyl-Oxalate Actinometer*
(Ergs/Cm.^{2}/Sec. $\times 10^4$)

Height of Lamps, Cm.	Burdick 1		Burdick 2		Small Cold Quartz		Large Cold Quartz	
	Titanium Cell	"Midultraviolet"	Titanium Cell	"Midultraviolet"	Titanium Cell	"Ultraviolet"	Titanium Cell	"Ultraviolet"
10	....	....	....	....	0.90	0.34	1.90	1.78
15	....	....	....	....	0.50	0.28	1.60	1.56
20	....	....	....	....	0.31	0.23	1.30	1.32
25	....	....	....	....	0.22	0.17	1.19	1.22
30	....	....	1.44	1.50	0.15	0.11	0.80	1.04
35	3.28	1.50	0.96	1.00	0.11	0.06		
40	2.54	1.00	0.78	0.70				
45	2.07	0.80	0.66	0.40				
50	1.54	0.50	0.51	0.20				
55	1.41	0.40	0.39	0.10				
60	1.21	0.20						

with the energy reaching the ones on the edges of the irradiated area. It is unlikely, however, that this introduced any considerable error or variation in the rate of tumor formation, since the mice were distributed at random each day in the irradiation compartments, and the number of such distributions over the seventy to one hundred and fifty irradiation days was sufficiently large for inequalities to cancel out. Energy calculations were based on the average amount of radiation falling on the whole area occupied by the mice. The mice received the full amount of radiation on the first day; it was not necessary to "build up" the level gradually.

In all groups receiving less than 23.1 $\times 10^7$ ergs per square centimeter of "complete" energy per day, the radiation was well tolerated. In a few groups the animals gained in weight, and some gave birth to young. Those on the lesser doses of radiation reared their young, occasionally even after the onset of cancer formation. On the higher levels of irradiation, however, the young were neglected. The mice were examined carefully at biweekly intervals. The most significant changes were observed in the ears in which the tumors were developing; the

3. The Purina dog chow contains the following ingredients in the percentages given: protein 20, fat 3, carbohydrate 56, ash 6 and water 15, with vitamins A and G added.

sequence of changes leading to tumor formation have been described elsewhere.[1a, b] The genetic background of the ABC or of the stock white mice did not appreciably alter the rate or incidence of tumor production.

With carcinogenic amounts of radiant energy, marked erythema occurred early in the experiment, but this subsided somewhat after three to four weeks. A ragged or stubby condition of the ears resulted when very large amounts of radiant energy were applied. This may have been due in part to direct destruction of tissue by the rays. A more probable cause was an induced irritation which led the animals to scratch their ears excessively. This damage of tissue, however, did not accelerate the production of tumors; tumors formed no faster in stubby than in intact ears.

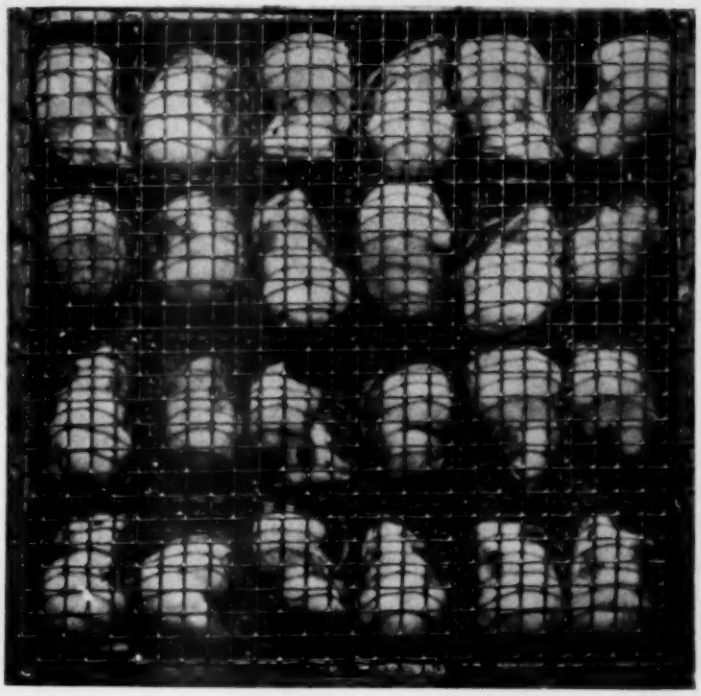

Fig. 2.—Irradiation cage. It has a sliding top.

While in general the time necessary for the production of a tumor varies inversely as the daily dose of radiant energy,[1a] this period was not reduced below two and a half months no matter how great the daily exposure. Neither the intensity of the energy nor the length of the daily exposure altered the rate of the production of tumors, provided the amount of energy applied per day was the same. Thus intense doses for short periods daily were as effective as mild intensities applied for longer periods each day.

EXPERIMENTS

Determination of Carcinogenic Wavelengths.—These experiments were performed serially, and the individual groups of any one series were directly comparable. Series I consisted of four groups of 24 mice

each which were irradiated sixty minutes a day seven days a week with the Burdick Uviarc lamp 2. Group 1 was exposed to the full energy of the lamp. Group 2 was exposed to energy passed through Corning filter 970, which excluded rays below 2,900 angstroms. For group 3 a filter of window glass was used, which excluded rays below 3,341 angstroms, and for group 4, Corning filter 352, which excluded most of the radiation below 4,700 angstroms. The transmissions of the different filters are given in figure 3.

The distance between the lamp and the irradiation cage was adjusted for each group so that all animals received 3.57×10^7 ergs of "ultraviolet" energy per square centimeter daily as measured by the oxalate actinometer. Even Corning filter 352 transmitted some rays capable of decomposing oxalate. The groups, therefore, all received the same total amount of energy over the range 2,000 to 4,500 angstroms, but

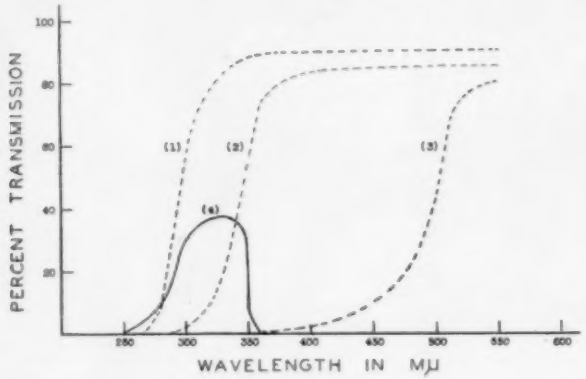

Fig. 3.—Transmission curves of the various filters: (*1*) Corning 970, (*2*) window glass, (*3*) Corning 352, (*4*) 986-$NiSO_4$.

the relative distribution of energy within that range had been altered by the filters.

The biologic response obtained depended on the filters used. Erythema was produced in groups 1 and 2, but none was induced in groups 3 and 4 throughout the experiment. No tumors appeared in the latter groups after one hundred and seventy days of irradiation. In the first two groups, on the contrary, the incidence of tumors ultimately reached 70 per cent. At one hundred and thirty-five days 44 per cent of group 1 (receiving the entire spectrum) and 55 per cent of group 2 (receiving only radiation of 2,900 angstroms and above) had tumors. The difference in results between groups 1 and 2 suggested that radiation shorter than 2,900 angstroms was either noncarcinogenic or less so than rays in the remainder of the "ultraviolet" spectrum (2,900 to 4,500 angstroms), for the total amount of "ultraviolet" energy given groups 1 and 2 had

been the same. Group 2, therefore, had received more energy of the range 2,900 to 4,500 angstroms than the control group and also showed a somewhat more rapid rate of tumor formation. Since in groups 3 and 4 no tumors developed, wavelengths of 3,341 angstroms and above must be regarded as noncarcinogenic. The range of carcinogenic wavelengths was therefore narrowed to the region between 2,900 and 3,341 angstroms. This conclusion was verified by further experimentation. Series II consisted of three groups of 24 mice each irradiated as follows:

(1) 0.29×10^7 ergs of "ultraviolet" energy per square centimeter from a small cold quartz lamp during thirty minutes on seven days of the week for two hundred and fourteen days. Most of this radiation was of wavelength 2,537 angstroms.

(2) 15.1×10^7 ergs of "complete energy per square centimeter (negligible "ultraviolet" energy) from a 300 watt tungsten Mazda lamp during thirty minutes on seven days of the week for one hundred and sixty-one days. This radiant energy was almost all visible and infra-red radiation.

(3) Grenz irradiation, 400 roentgens, 9 kilovolts, 8 milliamperes, for two minutes three times a week for forty-two weeks. The distance of irradiation was 10 cm. After forty-two weeks 7 survivors had received a total of 50,400 roentgens (5,040 ergs). This amount was not carcinogenic nor did it produce erythema. The dose given was obviously too small, although it was capable of causing a mild erythema of human skin.

In none of the animals in any of the groups did tumors develop, demonstrating that long waves, such as 4,100 angstroms and over, and the 2,537 angstrom band are noncarcinogenic.

The experiments with "short" ultraviolet rays were repeated, using larger amounts of energy.

Group 1 received 3.36×10^7 ergs of "ultraviolet" energy per square centimeter daily from the small cold quartz lamp. The irradiation was for sixty minutes seven days a week for a total of one hundred and seventy days. Even though a total of 571.2×10^7 ergs had been given, no tumors were produced. Only 0.5 per cent of this total radiation was "midultraviolet" energy.

Group 2 received 7.90×10^7 ergs of "ultraviolet" energy per square centimeter per day from the large cold quartz lamp. Irradiation was for sixty minutes six days a week for a total of one hundred and seventy-

three days. At the end of this period a total of $1{,}182 \times 10^7$ ergs per square centimeter had been given, and only one tumor was produced. The fact that 7 per cent, or 83.0×10^7 ergs of the "ultraviolet" rays consisted of "midultraviolet" energy may explain this carcinogenic activity. At all events, this level agrees closely with the amount of "midultraviolet" energy required for the production of cancer with the Uviarc lamps. At the end of one hundred and fifty-three days $1{,}027.0 \times 10^7$ ergs per square centimeter of "ultraviolet" radiation had been applied, which is almost ten times the amount of "midultraviolet" energy required for carcinogenesis in 50 per cent of the mice over a similar period, and yet not one tumor was produced (table 2). While

TABLE 2.—*Amount of Energy Necessary for Carcinogenesis (Continuous Exposure of Animals to Light)*

Mice		Amount of Irradiation					Initial Energy *			Average Energy		
				Daily Energy, Ergs/Sq. Cm. $\times 10^7$								
Series	Effective Total	Minutes per Day	Days per Week	"Complete"	"Ultraviolet"	"Midultraviolet"	Rays to First Tumor	Total Days Irradiated	"Midultraviolet," (Ergs/Sq. Cm. $\times 10^7$)	Days Until 50% Have Tumors	Total Days Irradiated	"Midultraviolet," (Ergs/Sq. Cm. $\times 10^7$)
I	18	60	7	13.85	2.52	0.51	106	106	53.5	135	135	69.0
	9	60	7	40.40	7.56	1.36	92	92	116.0	157	157	197.8
	16	30	7	20.02	3.78	0.63	106	106	66.4	132	132	83.0
	16	30	7	27.75	5.66	1.01	92	92	92.8	200	200	202.0
II	16	30	3	11.97	1.26	0.25	None	...	....	0	58	14.7
	11	30	6	11.97	1.26	0.25	None	...	....	0	116	29.4
	7	15	6	5.98	0.63	0.13	None	...	....	0	116	14.7
	21	15	6	11.97	1.26	0.25	219	116	29.4	0	116	29.4
III	23	30	6	15.50	2.48	0.97	75	65	63.0	164	140	134.5
	19	20	6	15.00	3.41	1.05	87	75	79.0	161	139	146.1
	19	30	6	18.34	3.32	1.09	90	78	85.3	170	146	159.6
	23	45	6	23.10	3.69	1.26	70	61	76.8	153	130	163.9
	22	30	6	22.46	5.08	1.47	72	61	89.9	186	161	203.8

* The initial energy is calculated as the "midultraviolet" portion of radiation necessary to produce the first tumor in any one group.

our results indicate that the 2,537 angstrom band has no carcinogenic activity, nevertheless the mice irradiated with energy from the cold quartz lamps showed a definite response to the radiation. The hair turned quite yellow, and the ears became red. However, the erythemal reaction could have been the result of wavelengths other than the 2,537 angstrom band.

Radiant Energy Necessary for Carcinogenesis.—In table 2 are listed the results of preliminary attempts to determine the amount of radiant energy necessary for tumor formation. The procedure adopted in all of these experiments was to irradiate the animals with the full spectrum of our Burdick quartz mercury lamps under the conditions previously described, and to continue the irradiation until tumors appeared. The desired energy levels were attained by adjusting the distance from the lamp to the animals. The various exposure times for the different

groups, as well as the amount of energy received daily per animal as determined by the three methods available, are listed in table 2. For purposes of discussion, the results are expressed as the amount of "midultraviolet" energy necessary for the appearance of tumors in 50 per cent of the animals in a group. The amounts of energy necessary for the appearance of the first tumors in the groups are likewise indicated.

Considerable variation was observed in the amounts of energy necessary for production of tumors in 50 per cent of the group, the values ranging from 67.2 to 236.0 $\times 10^7$ ergs over the irradiation period, the mean being 159.8 $\times 10^7$ ergs. There are, however, several reasons for favoring the lower rather than the higher figures. In the first place, excess ultraviolet radiation appears to cause harmful effects in mice quite independent of the processes which lead to tumor formation. On the higher doses of radiant energy, the animals lost weight, their physical condition was visibly poorer, they were less animated, and reproduction ceased. In such animals the rate of tumor formation was sometimes actually slower than in animals receiving less radiation and remaining in good physical condition. Furthermore, the ratios of "complete" to "ultraviolet" to "midultraviolet" energy varied somewhat in series I and III, depending on which lamp was used. Since there was some difference in the energy required for tumor production in these two series, it is possible that noncarcinogenic wavelengths of radiation may so alter the physiologic condition of the animals that the rate of tumor formation can be varied, even in the presence of equivalent "carcinogenic" amounts of radiant energy.

That the energy necessary for carcinogenesis is small is further indicated by the fact that a few tumors appeared relatively early in all groups. The "midultraviolet" energy necessary for the appearance of the first tumor in each group ranged from 29.4 to 115.9 $\times 10^7$ ergs over the irradiation period (table 2), the mean being 76.8 $\times 10^7$ ergs. These values do not necessarily represent the exceptional animal, although individual variation must, of course, be considered. As the irradiation was continued, most animals showed signs of irritation by frequent twisting and turning. The animals in which tumors developed most rapidly could therefore have been the more quiescent animals, i. e., those receiving more of the calculated amount of radiant energy.

A third reason for stressing the minimum rather than the average figures for energy requirements is the fact that the precancerous phase of ultraviolet ray tumors can be separated into two stages: the period of exposure to radiation and the latent period. This was demonstrated in the following ways: A group of 32 mice was irradiated with the Burdick mercury vapor lamp in such a way that 6.13 $\times 10^7$ ergs of "midultraviolet" energy per square centimeter was administered daily (table 3). Groups of 4 were then withdrawn from irradiation after four, five, six,

seven, nine, eleven, thirteen and fifteen weeks, but the periodic examination for tumors was continued. At least one tumor appeared ultimately in all groups irradiated for six weeks or more. The tumors appeared on the ears and were indistinguishable from those which resulted when irradiation was continued throughout the precancerous period. In general, the length of the "latent" period was inversely proportional to the length of the period of irradiation. Thus, in the group irradiated for forty-nine days it took two hundred and eleven days more for a tumor to appear; in the group irradiated for seventy-seven days the subsequent latent period was only sixty-three days (table 3). The experiments were repeated with both larger and smaller daily doses of

TABLE 3.—*Amount of Energy Necessary for Carcinogenesis (Limited Exposure of Animals to Light)*

		Amount of Irradiation			Appearance of Tumor		
Series *	Mice	Daily Energy ("Midultraviolet" Ergs/Sq. Cm. $\times 10^7$)	Total Days Irradiated	Tumors	Days from Start of Irradiation	Days After Stopping Irradiation (Latent Period)	Total Energy ("Midultraviolet" Ergs/Sq. Cm. $\times 10^7$)
I	4	0.63	35	None	...	...	22.2
	4	0.63	42	1	154	112	26.4
	4	0.63	49	1	260	211	31.1
	4	0.63	63	1	182	119	39.9
	4	0.63	77	2	140	63	48.3
	4	0.63	91	1	140	49	57.1
	4	0.63	105	1	140	35	65.9
II	6	0.55	36	None	...	...	18.1
	6	0.55	48	None	...	...	24.4
	6	0.55	63	None	...	...	31.9
III	24	1.00	39	1	135	90	42.0
	12	1.00	52	None	...	...	56.7

* Series I irradiated thirty minutes daily seven days a week; series II and III irradiated thirty minutes daily six days a week.

energy, with no essential difference in results. The total "midultraviolet" energy necessary for tumor formation under these conditions ranged from 26.4 to 66.0 $\times$ 10^7 ergs, most of the figures being under 42 $\times$ 10^7 ergs. While it should be emphasized that the ultimate tumor formation in these series did not exceed 25 per cent, it is nevertheless obvious that the determination of energy requirements for tumor formation is more accurate under these conditions than when irradiation is continued throughout the precancerous phase. The existence of a latent period in the development of human cancer is well known, particularly in cancers due to radium, roentgen rays and ultraviolet rays.

COMMENT

Our results indicate that the carcinogenic wavelengths of ultraviolet radiation definitely lie between 2,537 and 3,341 angstroms and probably lie between 2,900 and 3,341 angstroms. Since our source of radiant

energy was the mercury spectrum, this means that one or all of the lines at 2,967, 3,021 or 3,130 angstroms are active. It is quite likely that activity could be demonstrated for other wavelengths of radiation within this region but not present in the mercury spectrum. Studies with monochromatic light would no doubt yield interesting results. Roffo [4] appears to have made the only other effort to determine the wavelengths of the carcinogenic rays. He concluded that the active portion lay between 2,900 and 3,500 angstroms, although his experiments did not exclude wavelengths below 2,900. In our experiments there was no doubt about the ineffectiveness of the 2,537 angstrom band.

The inactivity of the 2,537 angstrom band is not the result of insufficient energy, for very large doses were given in our experiments, nor does it appear that the ineffectiveness can be explained by inadequate penetration. Bachem and Reed [5] found that 19 per cent of the 2,537 angstrom line was transmitted through the corneum of human skin, as compared with 34 per cent transmission for the wavelength 3,000 angstroms. The amount of radiant energy transmitted through the very thin corneum of the mouse is probably much greater than the amount for human skin, although the relative transmittancies for the two wavelengths may well be the same.

If we apply these figures directly to mouse skin and assume that 90 per cent of the "ultraviolet" energy from our cold quartz lamps was of the wavelength 2,537 angstroms, the amount of 2,537 angstrom radiation penetrating the skin can be calculated as follows:

7.9×10^7 ergs/sq. cm./day (from the large cold quartz lamp) $\times$ 19% = 1.39×10^7 ergs/sq. cm./day.

3.69×10^7 ergs/sq. cm./day (from the small cold quartz lamp) $\times$ 19% $\times$ 90% = 0.59×10^7 ergs/sq. cm./day.

Both figures are significantly higher than that for the energy which will produce cancer when "midultraviolet" radiation is used:

0.63×10^7 ergs/sq. cm./day (from the Uviarc lamp, table 2) $\times$ 34% = 0.21×10^7 ergs/sq. cm./day.

The amount of ultraviolet radiation necessary for carcinogenesis is, however, smaller than our figures indicate. Our calculations of the radiant energy applied are at best only an approximation of the amount which actually falls on the ear. Because of the twisting of the mice in their cages, we may estimate that only about 75 to 90 per cent of the applied energy reaches the surface of the ears. Reflection of light from the skin and transmission through the corneum to the basal cell layers involve other variables. Of the energy which reaches the ear surface, we are probably safe in assuming that at least 50 per cent is

4. Roffo, A. H.: Strahlentherapie **66**:329, 1939.
5. Bachem, A., and Reed, C. I.: Am. J. Physiol. **97**:86, 1931.

transmitted through the corneum. This is based on the fact that a 34 per cent transmission has been demonstrated through the much thicker and more pigmented human corneum.[5] If these corrections are considered, we find that of an average of 84×10^7 ergs per square centimeter of applied energy, only 31.5×10^7 ergs per square centimeter actually reaches the deeper layers. Since the area of the developing tumor is very small, it is clear that the amount of energy needed for carcinogenesis is also very small.

The carcinogenic wavelengths appear to be identical with part of the erythemal curve obtained by Coblentz, Stair and Hogue,[6] which reached its maximum effectiveness at about 3,000 angstroms. They also reported a second erythema-producing region around 2,500 angstroms. Lynch[7] tested the reaction of the skin of a child with xeroderma pigmentosum and demonstrated the skin to be most sensitive to radiations between 2,800 and 3,100 angstroms. Erythemal activity for human skin need not imply carcinogenic action in mouse skin. It appears, however, that the erythema produced in our experiments, with the cold quartz lamps, was largely due to the 2,537 angstrom band, but because of the presence of other wavelengths, this was not proved. Since this band did not induce cancer, it would appear that erythema and carcinogenesis may not necessarily be parallel phenomena.

SUMMARY

The carcinogenic wavelengths of the spectrum were found to lie between 2,900 and 3,341 angstroms. Wavelengths of 2,537 or 3,341 angstroms and above were noncarcinogenic. The carcinogenic wavelengths thus coincided in part with those most potent in the production of erythema. However, radiant energy with a wavelength of 2,537 angstroms produced erythema without inducing tumors.

Very little radiant energy was needed to initiate those changes which culminate in tumor formation, 63 to 84×10^7 ergs per square centimeter of effective "midultraviolet" radiation being adequate when given over a three month period.

While in general the length of the precancerous period varied inversely with the daily dose of radiant energy, the minimum time for the development of tumors appeared to be about two and one-half months. It was not necessary to irradiate the animals throughout the precancerous period. Once initiated, carcinogenesis proceeded without further exposure to radiant energy, and in isolated cases several months elapsed between the end of irradiation and the appearance of tumors.

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EFFECT OF HIGH PROTEIN DIETS ON EXPERIMENTAL ATHEROSCLEROSIS OF RABBITS

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When the first experimental atherosclerosis of rabbits was observed by Ignatowski,[1] in 1908, the changes were attributed to the animal protein in the diets, which included meat, milk and eggs. Subsequently, in 1912, Stuckey [2] demonstrated that the important factor in the animal food was not the protein but the lipids. In 1913 Anitschkow [3] succeeded in producing atherosclerosis with pure cholesterol, which was dissolved in vegetable oil to facilitate absorption. There has persisted, however, an impression that protein, especially animal protein, is a factor in the production of experimental atherosclerosis.

Several investigators have studied this problem. Newburgh,[4] in the course of an investigation of the effect of high protein diets on the kidneys of rabbits, observed atheromatous lesions in the aortas of 3 rabbits that had received a high casein diet for a year and in 1 rabbit that had consumed three to five egg whites daily for ten months. Untreated casein, however, contains considerable quantities of cholesterol. Subsequently Newburgh and Squier [5] and Newburgh and Clarkson [6] extended these observations to include a group of rabbits fed a diet rich in powdered beef. Newburgh and Clarkson expressed the belief that the beef protein was responsible for the atherosclerosis and hypercholesteremia observed and that the amount of cholesterol ingested with the meat (18 to 31 mg. per kilogram of body weight daily during eighteen to thirty-six weeks) was in itself insufficient. Unfor-

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1. Ignatowski, A.: Ber. d. mil. med. Akad., Petersburg **16**:174, 1908; Virchows Arch. path. Anat. **198**:248, 1909.
2. Stuckey, N. W.: Centralbl. f. allg. Path. u. Anat. **23**:910, 1912.
3. Anitschkow, N.: Beitr. z. path. Anat. u. z. allg. Path. **56**:379, 1913.
4. Newburgh, L. H.: Arch. Int. Med. **24**:359, 1919.
5. Newburgh, L. H., and Squier, T. L.: Arch. Int. Med. **26**:38, 1920.
6. Newburgh, L. H., and Clarkson, S.: Arch. Int. Med. **31**:653, 1923; J. Exper. Med. **43**:595, 1926.

tunately, the cholesterol fed in their meat-free control experiments was given as a dry powder in capsules, so that it probably was not absorbed so completely as the cholesterol in the meat. It has been well established (Duff [7]) that the amount of cholesterol required to produce atherosclerosis depends on the medium in which it is fed. Quantities such as those used by Newburgh when given in oil usually produce moderate atherosclerosis if fed for twelve weeks, followed by an equal rest period without cholesterol. Previously Kon [8] had observed atherosclerosis in rabbits on a diet containing dried liver. It was only with largely defatted liver that lesions failed to occur.

The occurrence of atherosclerosis was observed by Nuzum and co-workers [9] in rabbits fed a diet composed of oats, tomato, alfalfa and cod liver oil. Cod liver oil is an excellent source of cholesterol, and the latter was presumably the essential factor in the production of the lesions. A diet composed of soybean, tomato, alfalfa and cod liver oil, however, failed to produce atherosclerosis. This may indicate a true inhibitory effect of soybean on atherosclerosis. Unfortunately, however, Nuzum did not state whether equal amounts of cod liver oil were consumed with the two diets; the oil was given ad libitum, and no data on blood cholesterol levels are available to give an idea of the quantities absorbed.

No atherosclerosis was observed by Freyberg [10] in rabbits fed high protein diets containing soybean or gluten flour. An attempt to determine the effect of a diet rich in defatted casein was unsuccessful, as the rabbits refused the synthetic diet.

Duff [7] summarized the situation concerning the relationship of protein and atherosclerosis as follows: "The idea that the feeding of protein in conjunction with cholesterol promotes the development of experimental cholesterol arteriosclerosis remains to be substantiated by clearcut experimental evidence. I know of no data to indicate that the administration of sterol-free protein diets can produce arterial lesions similar to those of experimental cholesterol arteriosclerosis."

The lack of unanimity among previous experimenters made advisable a further study of this problem.

METHODS

A diet was devised characterized by an available protein content of 38 per cent (based on dry weight), the main source of the protein being casein. The casein was rendered essentially cholesterol free by the method of Sperry: [11]

7. Duff, G. L.: Arch. Path. **20**:81 and 259, 1935.
8. Kon, Y.: Verhandl. d. jap. path. Gesellsch., 1914, p. 105.
9. Nuzum, F. R.; Osborne, M., and Sansum, W. D.: Arch. Int. Med. **35**: 492, 1925. Nuzum, F. R.; Seegal, B., and Garland, R.: ibid. **37**:733, 1926.
10. Freyberg, R. H.: Arch. Int. Med. **59**:660, 1937.
11. Sperry, W. M.: J. Biol. Chem. **68**:357, 1926.

Commercial casein was continously extracted with hot 95 per cent alcohol for eight hours on three successive days. The casein, which at first had a strong tendency to cake, was allowed to stand overnight in cold 95 per cent alcohol before the regular extraction was begun. It was also removed from the percolator following each day's extraction, ground up carefully and allowed to dry overnight. Batches of extracted casein were pooled, and aliquot samples were analyzed for cholesterol as follows: Either 20 or 100 Gm. samples were saponified with approximately 25 per cent sodium hydroxide by heating six to eight hours on a steam bath. The saponified mixture was shaken out with purified petroleum benzine U. S. P. (petroleum ether). The purified petroleum benzine extracts were evaporated to dryness, redissolved in purified petroleum benzin and filtered into a weighing bottle. The amount of cholesterol in this nonsaponifiable fraction was determined by the method of Sperry,[12] with the results given in table 1.

TABLE 1.—*Cholesterol Contents of Batches of Casein Used in Experiments*

Batch of Casein	Sample Analyzed, Gm.	Cholesterol Content of Sample
A	20	None
B	20	None
C	20	None
D	20	None
E	100	0.08 mg.
F	100	Trace or 0.024 mg.
G	100	0.107 mg.

TABLE 2.—*Composition of Diets*

Ingredients	Number of Grams Fed per Day per Rabbit		
	Basic Diet *	Casein Diet *	Soybean Diet *
White flour	23.0	16.7	
Alfalfa leaf meal	20.0	16.7	20.0
Linseed oil meal	7.0	....	5.0
Ground carrots	50.0	50.0	50.0
Casein	....	16.7	
Soybean flour (Nusoy)	....	....	25.0
McCollum's salt mixture	0.5	0.5	0.5

* In the preparation of each diet the dry constituents were mixed and the carrots added together with water equivalent approximately to the total weight to make a moist mash.

The defatted casein was mixed with white flour, powdered alfalfa leaf meal, McCollum's salt mixture [13] and ground fresh carrots in the quantities given in table 2. Sufficient water (approximately an equal weight) was incorporated to make a moist mash.

The control, or basic, diet had approximately an equivalent caloric value but contained only 15 per cent of protein, derived from alfalfa leaf meal and linseed oil meal, together with white flour and carrots (table 2). In order to study the effect of a different form of protein, one free from objectionable traces of cholesterol, a second high protein diet was designed. This diet contained approximately the same amount of protein as the casein diet, but the chief source of the protein was

12. Sperry, W. M.: Am. J. Clin. Path. **8**:91, 1938.
13. McCollum, E. V., and Simmonds, N.: J. Biol. Chem. **33**:55, 1918.

soybean. Such a diet, while containing plant sterols, is devoid of cholesterol. Plant sterols are not absorbed in the animal body except, perhaps, in infinitesimal amounts (Schönheimer [14]). (The soybean product used was Nusoy, a white flour preparation containing 65 per cent protein, 3 per cent fat, 22.4 per cent carbohydrate [largely nonsoluble], 2.6 per cent ash and 7 per cent moisture. Attempts were made to use unrefined yellow soybean flour, but this caused diarrhea in some of the rabbits, presumably because of the higher content of soluble fermentable sugars.) To the white soybean flour were added alfalfa leaf meal, linseed meal, carrots and the salt mixture as indicated in table 2. More water was required in the preparation of this diet than in that of either of the others, since soybean flour absorbs a relatively large amount.

The number of grams of digestible protein, carbohydrate and fat in each diet and the caloric value of each diet are indicated in table 3.

TABLE 3.—*Food and Caloric Values of Diets* *

Diet	Protein		Carbohydrate		Fat †		Calories per Rabbit per Day
	Grams per Day	Percentage of Diet	Grams per Day	Percentage of Diet	Grams per Day	Percentage of Diet	
Basic	8.2	15	30	55	2.9	5	190
Casein	21.5	38	22	39	2.1	4	215
Soybean	22.0	39	19	34	1.6	3	200

* These calculations are from tables of total digestible ingredients as given by Henry and Morrison in their book "Feeds and Feeding" (Ithaca, N. Y., The Henry-Morrison Co., 1928) and are based on the dry weights of the diets.

† These figures do not include the cholesterol or the olive oil.

Cholesterol, purified by repeated recrystallization from alcohol, was administered to certain of the rabbits. To facilitate absorption, the daily dose was dissolved in 1 cc. of olive oil (heated when necessary) and was added to the top of each animal's daily ration. One cubic centimeter of olive oil alone was added to the ration of each control animal. (The olive oil was found on analysis to contain no cholesterol.)

Each rabbit was in an individual cage. It was fed the diet once daily and usually consumed all its food eagerly. No other food was given, but water was available. The animals thrived on the various diets, gaining weight during the experiments (100 to 500 Gm.) and when put to death were in an excellent state of nutrition.

At the end of the experiments the organs were fixed in Zenker's solution and stained with hematoxylin and eosin, and with special stains when indicated. The entire aorta was opened and rolled into a coil, fixed in a 1 : 10 dilution of solution of formaldehyde U. S. P., embedded in gelatin, and sections stained by a combination of hematoxylin,

14. Schönheimer, R.: Ztschr. f. physiol. Chem. **180**:32, 1929.

Weigert's elastic tissue stain and sudan IV. This method[14a] has been found useful in studying vascular changes. Blocks of heart, taken to include coronary arteries, were treated similarly.

After gross and microscopic examination, the degree of atherosclerosis of the aorta was recorded as +, ++ or +++. The significance of the grades may be stated as follows:

+ No lesion, or but a single, questionable one, was visible grossly, but there was microscopic evidence of intimal thickening and lipid infiltration, including anisotropic material.

++ A single small yellow plaque or several were grossly discernible in the arch of the aorta.

+++ Numerous plaques were seen in the thoracic portion of the aorta with, in some cases, an occasional plaque in the abdominal portion.

In order to detect more critically the effects of diet, the doses of cholesterol were kept well below that known to cause maximal atherosclerosis.

Chemical Determinations.—At approximately monthly intervals the plasma cholesterol of each rabbit was determined by the method of Sperry.[12] The Zeiss Pulfrich photometer was employed for measuring the cholesterol color values in the first part of the work, with the aid of Dr. Warren Sperry, who cooperated in making the determinations. Later determinations were done on a photoelectric apparatus designed by Dr. A. A. Weech, who made it available in the laboratories of the Babies Hospital. A certain number of analyses were also done for serum total nitrogen, nonprotein nitrogen, carbon dioxide–combining power and calcium.

Total nitrogen was determined by micro-Kjeldahl digestion and Pregl steam distillation as given in Peters and Van Slyke[15] with a modification suggested to us by Dr. Michael Heidelberger, of distilling the ammonia into a saturated boric acid solution and then titrating it directly with hundredth-normal hydrochloric acid. Nonprotein nitrogen was similarly determined on a trichloroacetic acid filtrate of serum (made with 1 cc. to 5 cc. of 10 per cent trichloroacetic acid). Plasma carbon dioxide–combining power was determined by the method of Van Slyke and Cullen.[16] Calcium was determined by the potassium permanganate method of Kramer and Tisdall.[17]

EXPERIMENT 1 (TABLE 4)

Relation of High Casein Diet to Atherosclerosis.—Ten young adult gray chinchilla rabbits, weighing around 2 Kg. and approximately equally divided as regards sex, were fed the basic diet for six months. During the first half of this period 60 mg. of cholesterol was added daily

14a. French, C.: Arch. Path. **30**:1243, 1940.

15. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry: Methods, Baltimore, Williams & Wilkins Company, 1932, vol. 2, pp. 527 and 530.

16. Peters and Van Slyke,[15] p. 298.

17. Kramer, B., and Tisdall, F. F.: J. Biol. Chem. **47**:475, 1921.

to the ration of each rabbit. During the second three months the diet alone was fed. Previous observations had established that during this rest period of three months hypercholesteremia usually persisted and cholesterol continued to be deposited in the vessels. Anitschkow [18] and Scarff,[19] among others, have shown that cholesterol arterial lesions do not disappear but persist for periods up to eight hundred and fifteen days after withdrawal of cholesterol from the diet.

One animal died of intercurrent infection. Of the aortas of the surviving 9 animals, 4 were normal grossly and microscopically; 2 presented minimal (+) sclerosis, while in 3 the lesions could be classified as ++ (table 4). The cholesterol content of the blood plasma rose in all but a single rabbit (fig. 1).

TABLE 4.—*Experiment 1: Relation of Casein in Diet to Sclerosis of Aorta*

Diet	Mg. Cholesterol Fed Daily	Rabbits	Amount of Sclerosis in Aorta *
Basic	60	10	0, 0, 0, 0, +, +, ++, ++, ++ (1 animal died)
Casein	60	5	0, ++, +++, +++, +++
		8 repeat experiment	0, 0, ++, ++, +++, +++, +++, +++
Casein	None	6	0, 0, 0, +, +, ++
		8 repeat experiment	0, 0, 0, +, +, +++ (2 animals died)
Basic	None	8	0, 0, 0, 0, 0, 0, 0, 0

* Each symbol represents the result in 1 animal. +, presence of a lesion was doubtful grossly but one was evident histologically; ++, one to several small atheromatous plaques were visible grossly; +++, numerous plaques were present.

Five rabbits were fed the high protein casein diet for a similar six month period, with 60 mg. of cholesterol being added daily during the first three months. A considerably greater degree of atherosclerosis was obtained in this small group, with +++ lesions in 3 and ++ lesions in 1. One animal was normal. The cholesterol level in the blood of these rabbits rose sharply.

Six rabbits received the high protein casein diet for six months without addition of cholesterol. An unexpected result was obtained. Lesions indistinguishable from those associated with cholesterol feeding were found in the aortas of 3 animals (+ in 2, ++ in 1). The plasma cholesterol increased above the usual upper limit of normal (100 mg. of total cholesterol per hundred cubic centimeters of plasma) in 5 of the group, attaining respectively 113, 120, 140, 153 and 158 mg. per hundred cubic centimeters of plasma.

Eight rabbits on the basic diet alone for six months were free of atherosclerosis, and their blood cholesterol levels did not exceed normal.

18. Anitschkow, N.: Verhandl. d. deutsch. path. Gesellsch. **23**:473, 1928.
19. Scarff, R. W.: J. Path. & Bact. **30**:647, 1927.

The apparent occurrence of hypercholesteremia and the cholesterol type of sclerosis in the rabbits on an essentially cholesterol-free casein diet led to a repetition of that portion of the work the following year.

Eight rabbits were fed the casein diet during six months, with addition of 60 mg. of cholesterol daily for the first three months. In 6 of the group atherosclerosis developed (+++ in 4, ++ in 2). The plasma cholesterol increased in all the animals, ranging from 109 to 431 mg. of total cholesterol per hundred cubic centimeters.

Eight rabbits received the casein diet alone for six months. Two died with intercurrent infections. The aortas of 3 of the remaining 6 presented atheromatous lesions (+ in 2, +++ in 1). An increase of blood cholesterol occurred in 5 of the 6, the total plasma cholesterol ranging from 130 to 279 mg. per hundred cubic centimeters, with the highest level in the rabbit having the most advanced sclerosis.

These results (summarized in table 4) seem to confirm the first, namely, that atherosclerosis and hypercholesteremia may be produced by feeding large amounts of casein to rabbits. The significance of the trace of cholesterol present in the casein is discussed later.

EXPERIMENT 2 (TABLE 5)

Effect of Soybean on Cholesterol Sclerosis.—The presence of irremovable traces of cholesterol in the casein high protein diet and the apparent exaggeration of cholesterol-induced sclerosis by the addition of casein to the diet suggested the substitution of some other form of protein for the casein. The soybean diet described was accordingly devised to have approximately the same protein content as the casein diet.

Eight rabbits were fed the soybean diet for six months. No atherosclerosis resulted, and there was no elevation of blood cholesterol.

Eight rabbits received the soybean diet for six months with the addition of 60 mg. of cholesterol daily during the first three months. Six survived the experimental period and were in excellent nutritional state when killed. Atheromatous changes were demonstrable in the aortas of only 2 of the 6, and in these it was minimal (table 5). This was less sclerosis than occurred in cholesterol-fed rabbits on either the basic or the casein diet. The blood cholesterol levels were also relatively low, the maximum for each rabbit in the group lying between 49 and 202 mg. per hundred cubic centimeters of plasma.

This apparent inhibitory effect of soybean on the deposition of cholesterol was further tested by giving larger amounts of cholesterol. Six rabbits were given the basic diet for six months with addition of 250 mg. of cholesterol daily during the first three months. (It was found necessary to heat the olive oil to effect solution of this quantity of cholesterol in 1 cc. of oil.) Atherosclerosis occurred in 4 of the 6 rabbits (+, ++, +++, +++) (table 5). Hypercholesteremia was rela-

tively marked with this dose of cholesterol, the maximum for each rabbit ranging from 240 to 800 mg. of total cholesterol per hundred cubic centimeters of plasma.

Six rabbits received the soybean diet during the same six month period with 250 mg. of cholesterol daily during the first three months. The vessels of 3 were normal. Atherosclerosis of the aorta was minimal in 2 animals and somewhat greater (++) in 1, although the blood cholesterol levels ranged as high as from 570 to 1,200 mg.

TABLE 5.—*Experiment 2: Relation of Soybean in Diet to Sclerosis of Aorta*

Diet	Mg. Cholesterol Fed Daily	Rabbits	Amount of Sclerosis in Aorta
Soybean	None	8	0, 0, 0, 0, 0, 0, 0, 0
Basic	60	9	0, 0, 0, 0, +, +, ++, ++, ++ (same as experiment 1)
Soybean	60	8	0, 0, 0, 0, +, + (2 animals died)
Basic	250	6	0, 0, +, ++, +++, +++
Soybean	250	6	0, 0, 0, +, +, ++
Basic	250*	6	+, +, +, ++, ++, ++
Soybean	250*	6	0, 0, 0, + (2 animals died)

* The duration of feeding was three months.

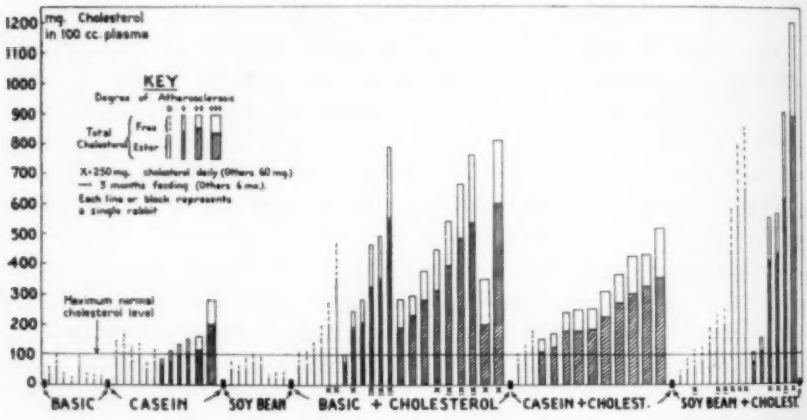

Fig. 1.—Degree of atherosclerosis and maximum plasma cholesterol levels.

When the feeding was carried out for only three months, a similar inhibition of atherosclerosis by soybean was apparent. All of 6 rabbits fed the basic diet plus 250 mg. of cholesterol daily for three months and killed at the end of that time had cholesterol plaques in the aorta (+ in 3, + + in 3), with blood cholesterol values from 470 to 800 mg. per hundred cubic centimeters of plasma. A similar group was fed the soybean diet plus 250 mg. of cholesterol daily; 4 survived, and only 1 exhibited any sclerosis (+) (table 5). The maximum total cholesterol in the plasma of each surviving rabbit was, respectively, 120, 230, 260 and 900 mg. per hundred cubic centimeters (fig. 1).

The chief findings in these several groups of rabbits are summarized in table 6. It seems that the presence of large amounts of casein in the diet tends to augment cholesterol atherosclerosis of the aorta, whereas the presence of large amounts of soybean flour tends to decrease the degree of sclerosis in comparison with a diet relatively poor in protein. The differences shown in the table are rendered even more significant by the fact that over half of the rabbits fed the soybean diet plus cholesterol (10 of 16), and over half of those fed the basic diet plus cholesterol (12 of 21) were given 250 mg. of cholesterol daily, while all of the animals on the casein diet plus cholesterol received only 60 mg. of the latter daily. Nevertheless the incidence and degree of sclerosis were highest in the last group.

TABLE 6.—*Summary of Feeding Experiments*

Diet	Cholesterol	Rabbits			Degree of Sclerosis of Aorta		
		Number Used	Number Sclerotic	Percentage Sclerotic	+	++	+++
Basic	None	8	0	0			
Casein	None	12	6	50	4	1	1
Soybean	None	8	0	0			
Basic	+	21	15	70	6	7	2
Casein	+	13	10	75	..	3	7
Soybean	+	16	6	35	5	1	

+, Presence of a lesion was doubtful grossly but one was evident microscopically; ++, one to several small atheromatous plaques were visible grossly; +++, numerous plaques were present.

OTHER PATHOLOGIC OBSERVATIONS

In addition to the atheromatous plaques, the aortas of one fourth of the rabbits presented areas of spontaneous medial degeneration. These occurred at random, without relation to any particular diet, and as a rule bore no anatomic relationship to the fatty lesions (fig. 2).

Atheromatous thickening of the intima of coronary arteries and of pulmonary arteries was occasionally observed in the rabbits with high blood cholesterol levels. Such lesions were much less frequent than those in the aorta and were not seen in the absence of the latter.

In view of numerous observations (summarized by Horn [20]) that diets high in protein can cause renal lesions, the kidneys from each animal were studied histologically. Beyond the occasional finding of the small pitlike depressions associated with patches of cortical scarring commonly seen in rabbits, no changes were observed, even in those few rabbits exhibiting transient azotemia.

All the rabbits had abundance of fat in the various body depots, and the adrenals were usually large, pale and well supplied with cortical lipid. A certain number of the livers were somewhat fatty, but this

20. Horn, H.: Arch. Path. **23**:71 and 241, 1937.

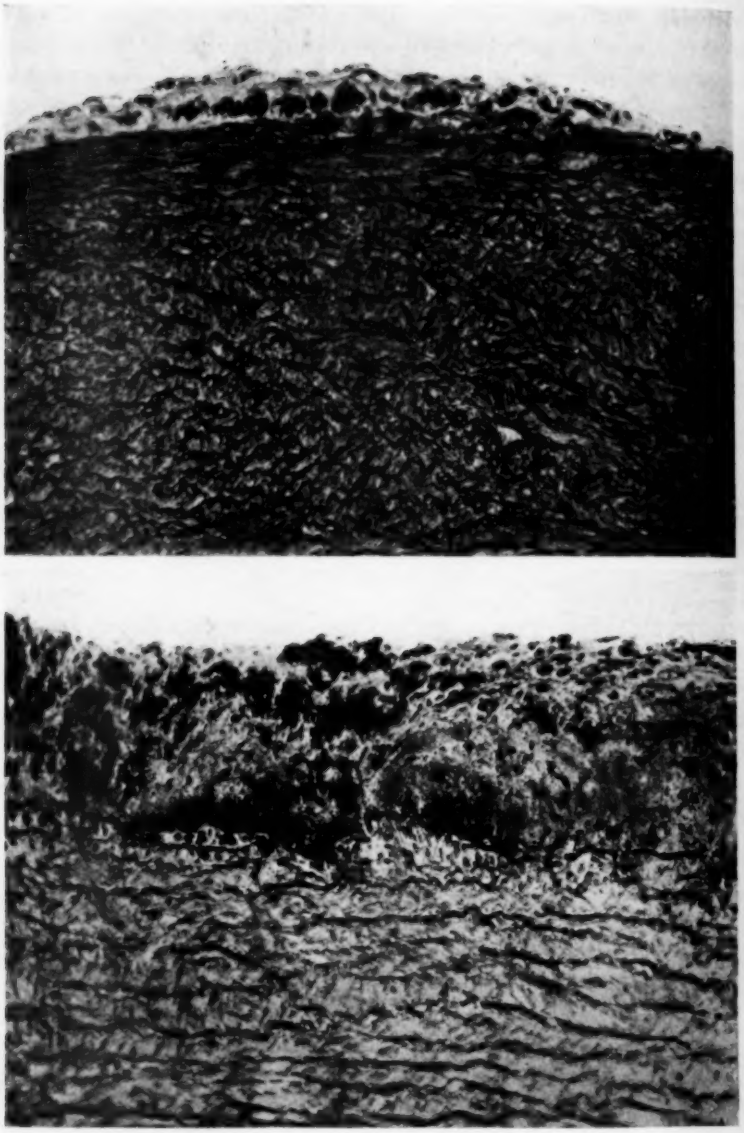

Fig. 2.—Upper: Rabbit 181. Soybean diet plus cholesterol. Lipid-laden phagocytes in intima. Sclerosis +.

Lower: Rabbit 187. Soybean diet plus cholesterol. Lipid-laden phagocytes in intima with diffuse fatty infiltration. Sclerosis + +. Sections stained with scarlet red, Weigert's elastic tissue stain and hematoxylin. Magnification, × 230.

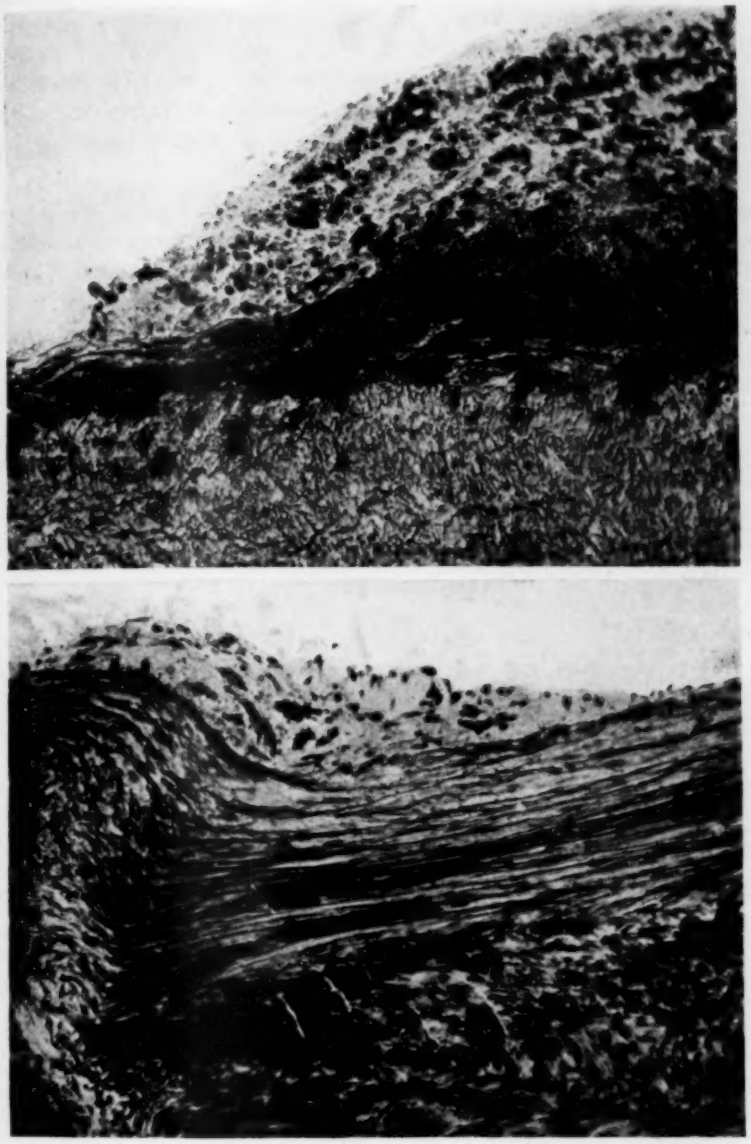

Fig. 3.—Upper: Rabbit 285. Casein diet without cholesterol. Edge of intimal fatty plaque. Diffuse infiltration of lipid into base of plaque and underlying media. Sclerosis + + +.

Lower: Rabbit 190. Soybean diet plus cholesterol. Atheromatous lesion of intima overlying area of spontaneous degeneration and sclerosis of media. Sections stained with scarlet red, Weigert's elastic tissue stain and hematoxylin. Magnification, × 230.

was not a constant finding in any one group of animals. No analyses of liver fat were carried out. Coccidiosis occurred only in occasional animals.

A majority of the thyroid glands from the rabbits were sectioned. No significant deviations from normal were observed, and no correlation between cellularity, size of acini and degree of atherosclerosis or type of diet was found.

BLOOD CHEMISTRY

Figure 1 gives in graphic form the relation of the degree of atherosclerosis and the maximum level of total cholesterol observed in the blood plasma of each rabbit. Such a chart gives, however, only a partial picture of the cholesterol metabolism of the animals. It fails to express the cholesterol levels over the six month (or three month) period of observation but gives merely a maximum value for each animal. In the case of the rabbits given cholesterol this value occurred as a rule toward the end of the period of cholesterol feeding. In some cases, however, the level continued to rise during the second half of the experiment, after cholesterol feeding had been discontinued. This would indicate further mobilization from such reservoirs within the body as the liver.

Figure 1 also indicates the ratio of combined to free cholesterol, the two making up the total cholesterol level. This ratio was fairly constant in rabbits that had not received cholesterol, averaging 1.26 ± 0.44 (standard deviation). With administration of cholesterol, the ratio of combined to free cholesterol gradually increased, and at the time of maximum cholesteremia it averaged 2.74 ± 0.61 (standard deviation). The type of diet fed with the cholesterol did not appear to affect this change in ratio.

The high casein diet did not influence the total nitrogen or the carbon dioxide–combining power of the serum. The total nitrogen varied between 6 and 8 per cent protein, and the carbon dioxide, between 55 and 75 volumes per cent. The normal serum calcium values covered a range of 12.3 to 14.8 mg. per hundred cubic centimeters; in the casein-fed animals there was a tendency for the values to rise slightly during the experiment, but the rise was not usually sufficient to be considered significant.

The reported effects of high protein diets on the kidneys of rabbits (Horn [20]) lent particular interest to the nonprotein nitrogen values. In the first part of the casein experiment (experiment 1) there were occasional animals that had values somewhat above normal on a single determination. This increase was never satisfactorily explained, however, as the nonprotein nitrogen values of all the rabbits during the last two months of the experiment were normal. Furthermore, during the

second part of the casein experiment and in the soybean experiment no elevation of nonprotein nitrogen was observed. These results are corroborated by the finding of normal kidneys in these animals.

COMMENT

The effect of a high protein diet on experimental cholesterol sclerosis of rabbits appears to depend on the protein fed. Defatted casein enhances the lesions; soybean flour diminishes them. Actually, casein without the addition of cholesterol appears capable of producing atherosclerosis.

The difference in the action of casein and soybean flour may depend on differences in composition of the respective proteins. Both casein and glycinin (the principal protein in soybeans) support growth. The chief differences in the amino acid composition of soybeans and of casein are: (*a*) Soybean protein contains 15.5 per cent of arginine, casein, 3.8 per cent; (*b*) soybean protein contains 0.7 per cent of valine, casein 7.9 per cent, and (*c*) soybean contains 1 per cent of glycine, casein 0.5 per cent (Schmidt;[21] Winton and Winton[22]). Whether arginine is capable of exerting a protective action against cholesterol sclerosis is not yet established, nor is information available on the influence of other quantitative differences between the two proteins.

Mention should be made of the amino acid methionine in view of the observations of Tucker and Eckstein[23] and of Channon and colleagues[24] that the lipotropic activity of proteins in preventing dietary fatty liver may depend on constituent amino acids, especially methionine. Baernstein's[25] analyses, however, indicate a methionine content of 3.53 per cent in casein and but 1.84 per cent in glycinin, the chief protein of soybeans. Cystine, on the other hand, has been found to increase the amount of fat in the liver,[23] but casein contains only 0.3 per cent of cystine, while glycinin yields 1.5 per cent.

Choline, in addition to reducing the deposition of fat in the liver of the animal on a high fat diet, has been observed by Steiner[26] to delay the occurrence of atherosclerosis in the cholesterol-fed rabbit and perhaps to accelerate absorption from cholesterol lesions. A dosage of 500 mg. daily was employed by Steiner. The choline content of the

21. Schmidt, C. L. A.: Chemistry of Amino Acids and Proteins, Springfield, Ill., Charles C. Thomas, Publisher, 1938, p. 217.

22. Winton, A. L., and Winton, K. B.: Structure and Composition of Foods, New York, John Wiley & Sons, Inc., 1932, vol. 1, p. 518.

23. Tucker, H. F., and Eckstein, H. C.: J. Biol. Chem. **121**:479, 1937.

24. Channon, H. J.; Loach, J. V.; Loizides, P. A.; Manifold, M. C., and Soliman, G.: Biochem. J. **32**:977, 1938.

25. Baernstein, H. D.: J. Biol. Chem. **97**:663, 1932.

26. Steiner, A.: Proc. Soc. Exper. Biol. & Med. **38**:231, 1938.

soybean diet was determined by the method of Fletcher, Best and Solandt [27] (acid hydrolysis, acetylation and titration, using a strip of smooth muscle) to be not over 1 part in 10,000. This corresponds to a daily choline intake per rabbit of approximately 10 mg. That this amount was sufficient to influence the deposition of cholesterol is problematic, particularly since the basic diet contained a similar amount of choline.

The apparent inhibition by soybean of the deposition of cholesterol might depend on an iodine effect, either directly or through the action of the thyroid gland in accelerating metabolism. Seel and Creuzberg [28] and Turner [29] found that potassium iodide tends to prevent cholesterol sclerosis in rabbits. Liebig [30] observed a similar effect of an organic iodide (diiodyl). Murata and Kataoka [31] had previously observed an inhibition of cholesterol sclerosis when thyroid gland was fed to rabbits which received "lanolin." [31a] This observation was extended by Turner and Khayat,[32] who found that the protective effect of potassium iodide was lost after thyroidectomy. It was not feasible to study the metabolism of the rabbits used in the present work. Histologic study of their thyroid glands failed to reveal any consistent differences between rabbits fed the soybean diet and those fed the casein diet or the basic diet. The iodine content of soybean is probably not constant, varying according to the soil on which the plant is grown. Soybean from several localities in north China was found by Adolph and Shen-Chao Ch'en [33] to contain 4 to 50 parts of iodine per billion parts of dry material. On the basis of the upper limit, the 25 Gm. of soybean flour fed daily to these rabbits contained approximately 0.001 mg. of iodine, an insignificant amount compared with the quantities previously found protective.

Of interest, too, is the increased alkalinity of urine and the increased plasma carbon dioxide capacity observed by Nuzum and co-workers [9] in rabbits fed soybeans. The effect of administration of alkali on the atherosclerosis of rabbits receiving cholesterol remains to be established. The reverse experiment, administration of an acid-forming substance, such as ammonium chloride, failed to accelerate the deposition of cholesterol (Jobling and Meeker [34]).

27. Fletcher, J. P.; Best, C. H., and Solandt, O. M.: Biochem. J. **29**:2278, 1935.
28. Seel, H., and Creuzberg, G.: Arch. f. exper. Path. u. Pharmakol. **161**:674, 1931.
29. Turner, K. B.: J. Exper. Med. **58**:115, 1933.
30. Liebig, H.: Arch. f. exper. Path. u. Pharmakol. **159**:265, 1931.
31. Murata, M., and Kataoka, S.: Verhandl. d. jap. path. Gesellsch. **8**:221, 1918.
31a. The article does not state whether the preparation used was hydrous or anhydrous wool fat.
32. Turner, K. B., and Khayat, G. B.: J. Exper. Med. **58**:127, 1933.
33. Adolph, W. H., and Ch'en, S. C.: Chinese J. Physiol. **4**:437, 1930.
34. Jobling, J. W., and Meeker, D. R.: Arch. Path. **22**:293, 1936.

The occurrence of hypercholesteremia and of atheromatous lesions of the aorta in rabbits receiving the casein diet alone was, of course, unexpected. The casein in this diet was presumably freed of cholesterol by continuous extraction with hot alcohol. Nevertheless, traces of cholesterol often remained (0.024 to 0.107 mg. per hundred grams of casein). This is in harmony with Freyberg's experience.[10] The total amount of contaminant cholesterol received by the rabbits during six months of feeding seems, however, too small to account for the results. A total of approximately 3 Kg. of casein was consumed by each rabbit. If all the casein fed contained 0.1 mg. of cholesterol per hundred grams of casein, the total possible amount of cholesterol ingested would have been 3 mg. This quantity is obviously inadequate to raise the plasma cholesterol from less than 100 mg. per hundred cubic centimeters (at the beginning of an experiment) to 150 mg. and even 280 mg. per hundred cubic centimeters, the plasma volume of a 2 Kg. rabbit being in the neighborhood of 100 cc.

The casein diet (by virtue of its plant components) also contained plant sterols (phytosterol), but Schönheimer[14] has established that phytosterol is not absorbed from the alimentary tract of the rabbit except possibly in minute amounts. The casein diet (as well as the other two) is, then, fundamentally sterol free. Herbivorous animals like the rabbit, which do not absorb plant sterols, must of necessity synthesize their tissue sterols from other elements in the diet. Apparently this synthesis is accelerated by casein but not by soybean protein in an equivalent amount. There result hypercholesteremia and atheromatous lesions in the blood vessels. Such a stimulation of cholesterol synthesis by casein might account, at least in part, for the enhancing effect of casein on cholesterol sclerosis noted in the rabbits fed cholesterol. In most of these animals well marked atherosclerosis developed, although they received a total of but 5.4 Gm. of cholesterol per animal during the experiment, an amount that added to the basic diet produced only a moderate degree of sclerosis. Of interest, however, is the discrepancy between this marked degree of sclerosis and the only moderately high levels of blood cholesterol (fig. 1), a discrepancy that suggests the existence in, or the production by, the casein of a toxic factor favoring deposition of cholesterol.

As stated by Duff,[7] previous data are lacking to indicate that sterol-free protein diets can produce atherosclerosis. Nevertheless the occurrence of atherosclerosis and hypercholesteremia in half of the rabbits fed the defatted casein diet alone cannot be explained as due to chance. ("Spontaneous" atherosclerosis has been encountered only once in this laboratory among several hundred rabbits not in contact with cholesterol, and other reports [Duff[7]] indicate an equal scarcity.) Nor can the results be fairly ascribed to possible contamination. The findings are

presented in the hope that they will prove a stimulus to other investigators to repeat the work, using, perhaps, a more efficient method of freeing casein of cholesterol.

The basic diet described in a foregoing section of this article, comprising white flour, alfalfa leaf meal, linseed meal, fresh carrots and salt mixture, is to be recommended as an excellent artificial diet for rabbits. Numerous attempts to feed rabbits a wide variety of artificial diets have been reported. In many instances the diets have been refused, and the animals have failed to thrive. The presence of alfalfa meal and of ground carrots in the diets used in the experiments described rendered these diets apparently highly palatable and at the same time allowed wide latitude in the distribution of other constituents.

SUMMARY

A high protein diet containing soybean flour diminishes the incidence and degree of experimental sclerosis in rabbits fed cholesterol.

A high protein diet containing defatted casein tends to increase the degree of experimental sclerosis in rabbits fed cholesterol.

In rabbits fed the high protein, defatted casein diet alone, hypercholesteremia and atheromatous lesions of the aorta may develop, indistinguishable from those produced by cholesterol.

CONCEPT OF FETAL ENDOCARDITIS

A GENERAL REVIEW WITH REPORT OF AN ILLUSTRATIVE CASE

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According to Rauchfuss,[1] the concept of fetal endocarditis was introduced in 1816, by Kreysig, who suggested that cases of pulmonary stenosis be studied in order to determine whether the stenosis was secondary to disease of the pulmonary artery and valves or whether it existed without traces of such disease. By the middle, and more toward the end, of the last century, fetal endocarditis was widely accepted as an entity and as an important cause of congenital cardiac anomalies. Supporters of this concept included Mönckeberg,[2] Sternberg,[3] Cruveilhier,[4] Herxheimer [5] and, in more recent times, Maude Abbott.[6]

The teaching of Rokitansky [7] that fetal endocarditis more commonly involved the right side of the heart, particularly the pulmonary valve, was paraphrased or modified to the effect that pulmonary atresia was usually of inflammatory origin [8] and that fetal endocarditis was rare on the left side of the heart.[9] Rauchfuss [1] expressed the opinion that fetal endocarditis occurring independently of developmental defects was no more uncommon in the left side than in the right side of the heart.

From the Western Pennsylvania Hospital Institute of Pathology; Ralph R. Mellon, M.D., director.

1. Rauchfuss, C.: Die angeborenen Entwicklungsfehler und die Fötalkrankheiten des Herzens und der grossen Gefässe, in Gerhardt, C.: Handbuch der Kinderkrankheiten, Tübingen, H. Laupp, 1878, vol. 4, pt. 1, pp. 12-154.

2. Mönckeberg, J. G.: Verhandl. d. deutsch. path. Gesellsch. **11**:224, 1907.

3. Sternberg, C.: Centralbl. f. allg. Path. u. path. Anat. **20**:198, 1909; Verhandl. d. deutsch. path. Gesellsch. **13**:198, 1909.

4. Cruveilhier, cited by Vaquez, H.: Diseases of the Heart, translated and edited by G. F. Laidlaw, Philadelphia, W. B. Saunders Company, 1924.

5. Herxheimer, G.: Missbildungen des Herzens und der grossen Gefässe, in Schwalbe, E.; Morphologie der Missbildungen des Menschen und der Tiere, 1910, Jena, Gustav Fischer, 1913, vol. 3, pt. 2.

6. (*a*) Abbott, M. E.: Congenital Cardiac Disease, in Osler, W., and McCrae, T.: Modern Medicine, Philadelphia, Lea & Febiger, 1927, vol. 4. (*b*) Abbott, M. E.: Personal communication to the author.

7. Rokitansky, cited by Rauchfuss.[1]

8. (*a*) Nagel, W.: Ein Beitrag zur Kasuistik über angeborene Herzfehler, Inaug. Dissert., Freiburg, Speyer & Kaerner, 1908. (*b*) Herxheimer.[5]

9. (*a*) Fischer, B.: Frankfurt. Ztschr. f. Path. **7**:83, 1911. (*b*) Nagel.[8a]

He expressed the belief that in many cases of developmental cardiac anomalies there was superimposed fetal endocarditis. Herxheimer [8] stated, "As a result of a thorough study of the literature, I would attribute the most important cause of (congenital) cardiac defects to *vitium primae formationis;* but fetal endocarditis may also cause cardiac malformations." According to Fischer,[9a] fetal endocarditis is a rare disease because of a special immunity of embryonal tissue. A more marked disagreement was indicated by Stohr,[10] who stated that the present view tended to assign the majority, if not all, congenital cardiac defects, primarily to anomalies of development.

Maude Abbott [6b] recently expressed herself as follows:

> I believe that in the majority of cases cardiac defects are due to a primary arrest of development of unknown origin but that in a certain smaller proportion of cases they are certainly the result of *fetal disease* involving either the myocardium or (less commonly) the endocardium. That fetal myocarditis is undoubtedly the cause in the group of the valvular atresias in question is a self evident fact, and it may have a still wider application for such myocardial changes based on toxic or infective agents circulating in the blood stream; and their setting in during very early embryonic life may be the cause of the arrest of development that underlies most cardiac defects. But we have no way of proving this, so that it is merely a guess.
>
> In cases of stenosis of the pulmonary or tricuspid valve in which the interventricular septum is closed and the lesion is limited only to the thickened and fused valve leaflets, the cause is certainly a fetal endocarditis setting in in late embryonic life, after the heart is fully formed. The cause of this fetal valvulitis might be any intercurrent infection, including rheumatic fever, though the latter so rarely attacks the mother during her pregnancy that it must be regarded as an infrequent cause.

In 1905 Ruge [11] collected 50 cardiac anomalies, of which 47 were considered caused by fetal endocarditis and 3 as probably of inflammatory genesis. In a more recent study of fetal hearts, Cappelli [12] found microscopic changes in 47 of 62 fetuses between the fifth and the ninth month which he regarded as indicative of fetal endocarditis. By way of contrast, Willer and Beck [13] wrote, in 1932, that doubt had arisen in the preceding ten years regarding the authenticity of the observations which had led to the concept of fetal endocarditis in the last half of the last century. This doubt was engendered by the dearth of microscopic studies, by the frequency with which the diagnosis had been made and by the almost complete lack of substantiating observations in the newer literature.

Analysis of the literature reveals that only 70 per cent of those abnormalities diagnosed as fetal endocarditis and 74 per cent of those

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10. Stohr, G.: Arch. Path. **17**:311, 1934.

11. Ruge, K.: Ueber angeborene Herzfehler mit besonderer Berücksichtigung der entzündlichen Stenose und Atresie der Aorta, Inaug. Dissert. Kiel, H. Fiencke, 1905.

12. Cappelli, E.: Sperimentale, Arch. di biol. **87**:129, 1933.

13. Willer, H., and Beck, L.: Ztschr. f. Kreislaufforsch. **24**:44, 1932.

considered developmental defects (including several which were not classified) were examined histologically.

The criteria for the diagnosis of fetal endocarditis were at first very loose and entirely macroscopic. In 1855 Dorsch [14] stated that the fibrous tissue found in the region of pulmonary stenosis was obvious evidence of myocarditis. Two years later Meyer [15] wrote that in all cases of pulmonary stenosis or atresia the obliteration of the pulmonary arterial lumen was without doubt the result of inflammation, since this condition could not be explained on any other basis. Because of the well known predilection of endocarditis for congenital defects, von Buhl [16] considered those cardiac defects occurring in early fetal life of developmental origin and those of later fetal life of endocarditic genesis. Rauchfuss [1] claimed that the absence of a developmental cardiac defect, such as a septal defect, was a criterion for inflammatory genesis, and a number of writers [17] based their diagnosis, at least in part, on this dictum. It was suggested by Letulle,[18] in 1914, that the term "fetal endocarditis" was a misnomer and that the term "isolated pancarditis" be substituted. This suggestion was based on the opinion that only healed, localized pancarditis which had left indelible scars could play the primary role in the subsequent maldevelopment of the heart. Some of the proponents [19] of fetal endocarditis have emphasized the resemblance, often striking, of certain cardiac defects to postnatal rheumatic valvulitis. The macroscopic criteria for the recognition of fetal endocarditis, according to these authors, are contractures, smooth or warty thickenings or adhesions of the valve leaflets, and endocardial opacities. On the other hand, Mönckeberg [20] wrote that verrucose vegetations on altered valves are not indicative of fetal endocarditis, since such vegetations may have formed secondarily as a result of superimposed infection.

Fischer,[9a] the first to emphasize the necessity of microscopic study, accepted only 2 of the cases reported up to 1911 as representing fetal endocarditis, because of the lack of histologic confirmation. His criteria

14. Dorsch, G.: Die Herzmuskelentzündung als Ursache angeborener Herzcyanose, Inaug. Dissert. Erlangen, Junge u. Sohn, 1855.

15. Meyer, H.: Virchows Arch. f. path. Anat. **12**: 497, 1857.

16. von Buhl: Ztschr. f. Biol. **16**:215, 1880.

17. (*a*) Niemyska, W.: Ueber Endocarditis parietalis congenita, Inaug. Dissert, Zurich, Leemann & Co., 1913. (*b*) von Zalka, E.: Frankfurt. Ztschr. f. Path. **30**:144, 1924. (*c*) Eakin, W. W., and Abbott, M. E.: Am. J. M. Sc. **186**: 860, 1933. (*d*) Farber, S., and Hubbard, J.: ibid. **186**:705, 1933. (*e*) Wesson, H. R., and Beaver, D. C.: J. Tech. Methods **15**:86, 1935.

18. Letulle, M.: Presse méd. **22**:432, 1914.

19. Willer and Beck.[18] Farber and Hubbard.[17d]

20. Mönckeberg, J. G.: Die Missbildungen des Herzens, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2.

for the diagnosis of fetal cardiac inflammation were extensive necrosis and calcifications of the myocardium, the presence of lymphocytes, numerous dilated capillaries, cellular connective tissue scars and giant muscle nuclei in the myocardium, as well as aggregations of leukocytes in capillary lumens. Although other writers [21] have agreed that microscopic confirmation is a necessity, it has been admitted [22] that such cytologic changes have not been consistently found in cases in which, nevertheless, fetal endocarditis has been diagnosed. Suvalischin (quoted by Pototschnig [22a]) attempted to brush aside the obvious contradiction between diagnosis and objective findings with the statement that, because of the great regenerative capacity of embryonal valvular tissue, all traces of inflammation could disappear.

In 1890 Klipstein [23] wrote that connective tissue scars in the myocardium, cloudy swelling, fatty degeneration of the endocardium and valvular deformities, such as contractures, adhesions and thickenings, were not necessarily proof of inflammation. Ribbert [24] pointed out that cardiac ostiums deformed through maldevelopment may show changes, i. e., smooth or nodular thickening of the valves, similar to those in fetal endocarditis. The ground glass appearance of the parietal endocardium in congenital stenosis of the pulmonary valve was considered by Keith [25] as caused by embryonic rather than inflammatory tissue. Similar tissue was present in the deformed valves. In a later Hunterian lecture he said, "I am convinced that these lesions are not the result of foetal endocarditis for the following reasons:

"It is impossible to conceive endocarditis giving rise to the great majority of the malformations which are here described."

Other reasons given were that there was no scar tissue in the neighborhood of the lesions except where there was postnatal superimposed infection and that malformations were present in other parts of the body.

A different opinion was expressed by Mönckeberg,[20] who stated that microscopic inflammatory changes were significant because they were not seen in even the most severe malformations. In support of the inflammatory concept, Maude Abbott [6a] wrote, "Careful investigation of the myocardium in most cases of atresia of the orifices will reveal patchy, yellow-gray areas visible to the naked eye, which microscopically show all stages of degeneration, vacuolation, fatty change, and even, in

21. (*a*) Fischer, B.: Verhandl. d. Gesellsch. deutsch. Naturf. u, Aerzte **80** (pt. 2): 43, 1908. (*b*) Bellet, S., and Gouley, B. A.: Am. J. M. Sc. **183**:458, 1932. (*c*) Stohr.[10] (*d*) Farber and Hubbard.[17d]

22. (*a*) Pototschnig, G.: Ztschr. f. ang. Anat. **4**:234, 1918. (*b*) Vaquez.[4]

23. Klipstein, L.: Arch. f. Kinderh. **11**:361, 1890.

24. Ribbert, H.: Die Erkrankungen des Endokards, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2.

25. Keith, A.: Lancet **2**:359, 519, 1909.

a case under the writer's personal observation, calcification with cellular infiltration, and a diffuse fibrosis. . . ."

It is a singular as well as noteworthy fact that, as indicated by Ribbert,[24] so-called "fetal endocarditis" has always been encountered in the healed stage only and that an active or acute form of this condition has never been seen. He also stated that he had failed to find a single case of endocarditis in 1,000 autopsies on children less than 3 years old. De Vecchi [26] wrote that Holt also had found no case of endocarditis in the course of 1,000 autopsies on infants under 3 years of age. However, several clinicians, in 1893 and 1906, according to de Vecchi,[26] found valvular disease in 4 to 5 per cent of children under 10 years of age. Such figures would suggest that in all probability too much emphasis was placed on cardiac murmurs per se.

A more formidable argument against the rarity of endocarditis in infancy is found in the observations of Cappelli,[12] who noted microscopic changes interpreted as valvular endocarditis in many fetal hearts. However, the significance of these observations appears dubious in view of the frequency, 75.8 per cent of 62 unselected cases, with which the lesions were found. Inasmuch as the histologic valvular changes described by de Vecchi [26] in infants and children were similar in many respects to those described by Cappelli,[12] the question of their significance may be raised here as well.

A careful analysis of the cases reported in the literature reveals that myocardial scars or myocardial fibrosis, stressed as prerequisites for the diagnosis of fetal endocarditis, was noted in 78 per cent of the cases listed as instances of fetal endocarditis. On the other hand, this lesion has also been found in 38 per cent of cases considered as instances of noninflammatory changes. A number of writers [27] noted that the papillary muscles showed the most marked lesions in "fetal cardiac inflammations." These observations may be matched by reports [28] of similar findings in hearts considered to show noninflammatory alteration.

Calcific deposits in the myocardium, also considered a criterion of inflammation, have been frequently recorded not only in cases accepted as instances of defects inflammatory in origin (44 per cent) but also in those considered instances of noninflammatory change (13 per cent). Fischer [9a] regarded this calcification as due to toxic necrosis of the myocardium or as secondary to fatty degeneration of the muscle fibers. Pototschnig,[22a] on the other hand, thought the calcium deposits were a

26. de Vecchi, B.: Arch. Path. **12**:49, 1931.

27. (*a*) Donat, R.: Frankfurt. Ztschr. f. Path. **53**:120, 1939. (*b*) Fischer.[9a] (*c*) Stohr.[10] (*d*) Fischer.[21a]

28 (*a*) Jacobsthal, H.: Virchows Arch. f. path. Anat. **159**:361, 1900. (*b*) Kockel, R.: Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte **80**: (pt. 2) 39, 1908. (*c*) Pototschnig.[22a]

dystrophic form of calcification derived from connective tissue and entirely independent of myocardium.

Vacuolar and fatty degeneration of the myocardium have been noted in hearts showing defects classified as inflammatory [29] and also in those showing changes considered noninflammatory.[30] Focal myocardial necrosis in fetal endocarditis was seen by several writers.[31] Giant myocardial nuclei were described by Fischer [32] only. Lymphocytes, which one might expect to find frequently in healed inflammations, have been described only six times in cardiac conditions diagnosed as fetal endocarditis [33]; but similar findings have been noted also in conditions not so diagnosed.[34]

Occasional mention of the presence of new, dilated or excessive numbers of capillaries in valvular or myocardial tissue has been made in support of an inflammatory origin.[35] The aggregation of leukocytes in capillaries, considered an important criterion of inflammation (acute ?) by Fischer,[32] was noted by only one other author.[10] Complete or partial obliteration of myocardial vessels was recorded by Letulle,[18] who regarded this finding in a 22 year old woman as evidence of pancarditis. Such vascular changes were also seen by Roberts,[36] who considered them as evidence of prenatal inflammation.

Three references to the presence of fresh acute valvular vegetations have been found in the literature. Two [37] of these refer to Klipstein's [23] report, in which the following sentence appears to have been misconstrued as descriptive of a vegetation: *Die Noduli Aranti bilden einen festen hirsekorngrossen rothen Körper.*

The third reference [27a] described thrombi which contained centers of leukocytes and were adherent to the mitral valve. No mention was made of the state of the endothelium underlying the thrombi or of the presence or absence of bacteria.

Valvular excrescences, often termed vegetations, were noted in 11 of 53 cases designated as cases of fetal endocarditis (20.8 per cent) and

29. Fischer.[9a] Willer and Beck.[13] Jacobsthal [28a] Kockel.[28b]

30. (*a*) Loeser, A.: Virchows Arch. f. path. Anat. **219**:309, 1915. (*b*) Dissmann, E.: Frankfurt. Ztschr. f. Path. **43**:476, 1932. (*c*) Pototschnig.[22a]

31. Fischer.[9a] Stohr.[10] Fischer. [21a]

32. Fischer.[9a] Fischer.[21a]

33. Willer and Beck.[13] von Zalka.[17b] Farber and Hubbard.[17d] Donat. [27a] Jacobsthal.[28a]

34. (*a*) Boger, A.: Beitr. z. path. Anat. u.z. allg. Path. **81**:441, 1929. (*b*) Stiassny, S.: Centralbl. f. allg. Path. u. path. Anat. **12**:27, 1901. (*c*) Tow, A.: Am. J. Dis. Child. **42**:1413, 1931.

35. Stohr.[10] Willer and Beck.[13] von Zalka.[17b] Pototschnig.[22a] Kockel.[28b]

36. Roberts, J. T.: Am. Heart J. **12**:448, 1936.

37. Ruge.[11] Bellet and Gouley.[21b] The latter authors make mention of "Klepstein" but the reference is to Klipstein.

also in 4 of 23 cases in which this diagnosis was not made (17.4 per cent). In the few instances in which these valves were examined histologically, these excrescences were described as composed of embryonic connective tissue. In no instance was there unequivocal evidence of inflammation. Foci of lymphocytes were noted in the valve in 1 case only.[13] Thickening and hyalinization of the aortic and mitral valves were described in 1 case.[17d] In another case [17e] the aortic valve was characterized as "the seat of extensive fibrosis."

Considerable attention has been paid within the last twenty years to those cardiac anomalies characterized by opaque, glistening white or yellow-white thickening of the parietal endocardium with or without valvular deformity. These lesions have been classified as fetal inflammations by some investigators and as developmental defects by others.

Although the incidence of parietal endocardial sclerosis is the same (70 per cent) in the cases classified as instances of fetal endocarditis as in the cases not so diagnosed, in the latter group this condition was frequently independent of valvular lesions, while in the cases termed instances of fetal endocarditis the parietal endocardial sclerosis was usually associated with stenosis or atresia of the aortic or pulmonary valves.

Becker [38] described the presence of intracellular hemosiderin, fibrin remnants and calcium deposits in the thickened parietal endocardium and consequently assumed a thrombotic origin. Loh [39] objected to the thrombogenic theory because in most cases of congenital endocardial fibrosis the process was too diffuse and too uniform, and furthermore, no hemosiderin was demonstrable.

Pototschnig [22a] was the first to suggest that the endocardial sclerosis was a simple noninflammatory hyperplasia of fibroelastic tissue. Ariel,[40] Dissmann,[30b] Loh,[39] Straus [41] and Eakin and Abbott [17c] expressed similar opinions. Some investigators [42] thought the hyperplasia was caused by overstretching of the endocardium and made mention of experiments by Dewitzky,[43] who produced endocardial fibrosis in rabbits by injecting epinephrine. Additional support for this theory was drawn from the work of Hübschmann,[44] who observed endocardial sclerosis in dilated hearts of patients dead from diphtheria. Mönckeberg [20] noted that sclerosis of the parietal endocardium with calcification and atherosclerotic

38. Becker, K.: Jahrb. f. Kinderh. **84**:64, 1931.
39. Loh, H.: Beitr. z. path. Anat. u.z. allg. Path. **101**:253, 1938.
40. Ariel, M. B.: Virchows Arch. f. path. Anat. **277**:501, 1930.
41. Straus, F.: Zentralbl. f. Gynäk. **54**:1235, 1930.
42. Straus,[41] Loeser.[30a]
43. Dewitzky, W.: Frankfurt. Ztschr. f. Path. **9**:82, 1912.
44. Hübschmann, P.: München. med. Wchnschr. **64**:73, 1917.

changes was common in aortic stenosis and expressed the belief that this was caused by circulatory changes.

Although the proponents of the fetal endocarditis concept are agreed that the fetal inflammation is derived from a maternal infection, many of them have failed to mention the existence of maternal illness during the puerperium, while others have been forced to admit its absence anamnestically. Several writers attributed fetal endocarditis to maternal influenza [45]; one, to bronchitis,[28b] and another, to "fever." [46] Niemyska,[17a] in the face of a history negative for maternal illness in her 2 cases, assumed the occurrence of angina or bronchitis which escaped notice. Abbott [6] expressed the belief that congenital syphilis or other infections carried in the coronary stream play the etiologic role. In 2 of 4 cases tabulated by Farber and Hubbard,[17d] the mothers were listed as cardiac patients. Altogether, there were 7 instances in which the existence of maternal illness was noted and 7 cases in which its absence was specifically recorded; in the remaining 39 cases [47] (74 per cent) no mention of either the presence or the absence of maternal illness was made.

Recently in the Institute of Pathology of the Western Pennsylvania Hospital a newborn infant was examined post mortem, whose heart possessed all the gross features and satisfied most of the microscopic criteria of fetal endocarditis. Certain histologic changes in this heart, hitherto not mentioned, suggested a new theory of noninflammatory origin which may be applicable to many of the congenital cardiac defects designated as fetal endocarditis.

REPORT OF CASE

A newborn boy, weighing 3,180 Gm., was born of a 21 year old white primagravida, a former nurse. The pregnancy was entirely normal except for slight vaginal bleeding during the first month. There was no history of any maternal infection during the pregnancy, and a careful examination, including serologic tests, after delivery established that the woman was in perfect health.

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45. Farber and Hubbard.[17d] Fischer.[9a]

46. Püschel, E.: Arch. f. Kinderh. **114**:1, 1938.

47. Bardeleben: Virchows Arch. f. path. Anat. **3**:305, 1851. Dilg, J.: ibid. **91**:193, 1883. Wolff, B.: Congenitale Aortenstenose, Inaug. Dissert., Berlin, G. Schade, 1899. Summons, W. H.: Intercolon. M. J. Australasia **11**:65, 1906. Ganeff, N.: Ueber angeborene Stenose und Atresie der Aorta durch fötale Endokarditis, Inaug. Dissert., Würzburg, C. J. Becker, 1910. Weill, E.; Delore, B., and Delore, P.: Lyon méd. **133**:308, 1924. Feldmann, R.: Jahrb. f. Kinderh. **150**: 138, 1937. Jost, J.: Zur Casuistik der angeborenen Fehler des linken Herzens, Inaug. Dissert., Giessen, Münchow, 1896. Philpott, N.: Ann. Int. Med. **2**:422, 1928. Diamond, M.: Arch. Path. **14**:137, 1932. Dolgopol, V. B.: J. Tech. Methods **13**:100, 1934. Colarizi, A.: Cuore e circolaz. **20**:66, 1936. To this list should be added reports of such cases already cited in the foregoing footnotes.

The obstetrician (Dr. James N. Stanton) performed a low forceps delivery and considered the infant healthy, although a respiratory grunt was noted. On the fourth day the baby suddenly became cyanotic, respiratory difficulty developed, and the baby died within twelve hours.

At autopsy the body was that of a full term, well developed newborn boy, 44 cm. long and 3.1 Kg. in weight. With the exception of a cardiac anomaly, diffuse hemorrhage throughout the pulmonary alveoli and the presence of fresh fluid blood in the tracheobronchial tree and in the stomach, no striking abnormality was present.

The heart weighed 23.0 Gm., was moderately enlarged and measured 43 mm. along the longitudinal, and 35 mm. along the transverse, axis. The apex of the heart was formed entirely by the right ventricle, and the apex of the left ventricle was situated 19 mm. above that of the right. Section disclosed a much larger than normal right ventricle, the wall of which measured 4.5 to 5 mm. in thickness, and a diminutive left ventricular chamber, measuring 4 by 7 by 10 mm., with a wall thickness of 6 to 7 mm. The endocardium of the left ventricle, 0.5 to 1.5 mm. thick, was composed of a dense, glistening white tissue, on the surface of which a few trabeculae carneae were vaguely suggested by occasional minute punctate or linear depressions. The endomyocardial border was sharply defined but irregular because of coarse endocardial bands extending vertically into the myocardium. The papillary muscles were quite small, and many chordae tendineae inserted directly into the ventricular wall. The mitral valve presented a funnel-shaped deformity, caused by fusion of the leaflets, which were thickened and opaque, glistening white. The ring measured 22 mm. in circumference, while the free edge was only 10 mm. in length. The chordae tendineae were markedly thickened and retracted. The entire valve bore a striking resemblance to the typical rheumatic mitral stenosis of postnatal life.

The left auricle was considerably enlarged, and its endocardium showed a number of narrow bands of thick, milky white tissue extending from the mitral ring and coursing irregularly to end indeterminately in the normal auricular endocardium. The aortic valve had only two cusps, and these were irregularly and nodularly thickened and were adherent to each other (fig. 1). The endocardium of both valves was everywhere intact, and vegetations were absent.

The enlarged right ventricle showed prominent, rotund trabeculae carneae and papillary muscles and was lined by a thin normal endocardium. The tricuspid and the pulmonic valves were normal. The right auricle was large, the pectinate muscles robust and the lining endocardium normal except for one narrow band of thickened endocardium which extended up from the tricuspid ring. The foramen ovale was anatomically patent but functionally closed (5 by 7 mm.). A minute blood cyst was present on the tricuspid valve, and a similar one was also found on the mitral valve. There was no septal defect. The coronary orifices and vessels were normal.

The aorta was definitely narrowed at the root, measuring 11 mm. in circumference, while in the thoracic region the circumference was 13 mm. In contrast, the ductus arteriosus was large and widely patent, measuring 18 mm. in circumference. Other measurements were as follows:

Aortic ring	11 mm. in circumference
Pulmonic ring	26 mm. in circumference
Tricuspid ring	46 mm. in circumference
Left auricular wall	1.5 mm. thick
Right auricular wall	1.0 mm. thick

Microscopic Observations.—The significant changes were limited to the parietal endocardium and the papillary muscle of the left ventricle. The greatly thickened parietal endocardium consisted of a dense collagenous tissue containing an abundance of elastic fibrils, which were arranged parallel to the free surface. The

Fig. 1.—Photograph of heart showing diminutive left ventricle lined by thickened endocardium, the deformed, fused, bicuspid aortic valve and the thickened, stenotic mitral valve; × 3.5.

endomyocardial junction was irregular because of the penetration of coarse trabeculae of endocardial tissue into the myocardium. Relatively few adult connective tissue nuclei and no inflammatory cells were present in the endocardium. Likewise,

vessels were few but normal and were situated almost exclusively near the myocardium, surrounded by thick collars of fibroelastic tissue. The endocardial surface was smooth and presented no interruptions for openings of arterioluminal or thebesian vessels (fig. 2 *A*).

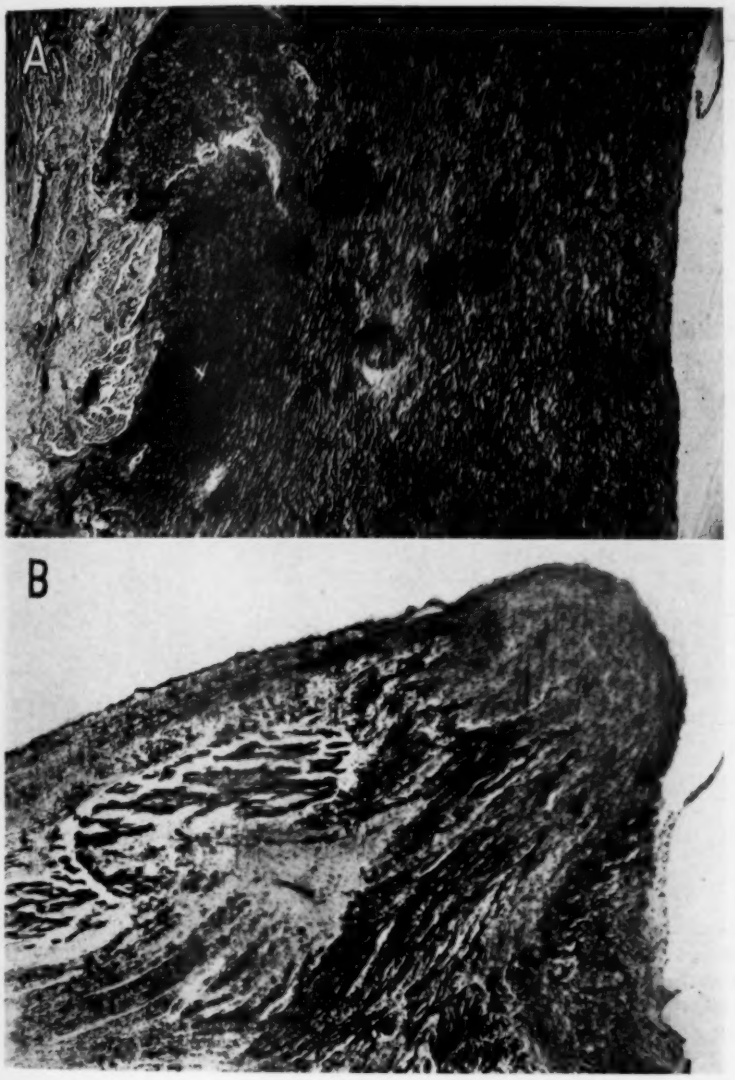

Fig. 2.—*A*, photomicrograph of sclerotic parietal endocardium showing at the right uninterrupted, smooth free surface, relative avascularity and abundance of elastic fibers. Weigert and Van Gieson stain; × 48. *B*, photomicrograph of papillary muscle showing fibrous replacement of cardiac muscle, calcification and (in central triangular lighter area) deposits of colorless crystals. Hematoxylin and eosin; × 39.

The myocardium was everywhere normal except for extensions of endocardial fibrous tissue and extensive fibrosis of the diminutive papillary muscle. Here an acellular hyaline collagen replaced much of the muscle. A large irregular mass of calcific material, as well as masses of refractile unidentified crystalline material, was present in the connective tissue (fig. 2 *B*). Inflammatory cells were absent here also. No degeneration or necrosis of muscle fibers was present, and scarlet

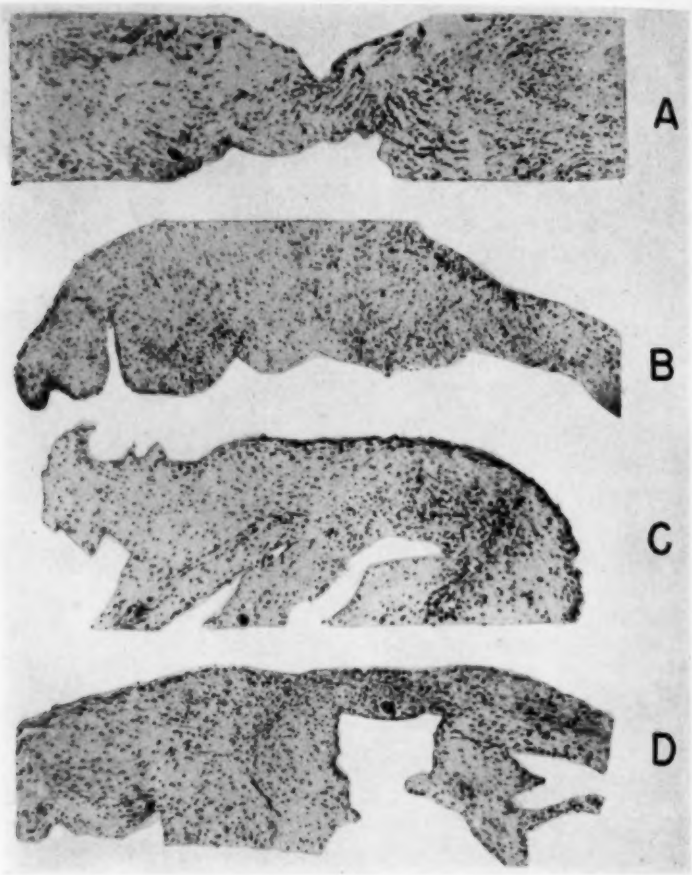

Fig. 3.—Photomicrographs of heart valves showing similarity of architecture and absence of inflammatory tissue: *A*, aortic valve; *B*, pulmonic valve; *C*, mitral valve; *D*, tricuspid valve. Hematoxylin and eosin; × 44.

red and osmic acid stains showed no fat in the endocardium or myocardium. Hemosiderin was not found. Vessels were normal.

The thickened mitral and aortic valve leaflets were composed of a loose connective tissue made up of interlacing fine fibrillae and a moderate number of branching plump fibroblasts (fig. 3). A small number of more dense spindle-shaped nuclei of more adult fibroblasts were also present. In addition, there were occasional isolated small round nuclei resembling lymphocytes, but these cells

appeared to be cross sections of mature fibroblastic nuclei. Vessels in the valves were not unusual in number or appearance. The tissue, except for its thickness, resembled closely that of a normal valve.

COMMENT

It is apparent that most, if not all, of the conditions which at one time or another were regarded as pathognomonic for fetal endocarditis, including the supposedly rigid microscopic criteria set up by Fischer,[32] are to be found in cardiac anomalies which, after study and consideration of the endocarditic concept, were diagnosed developmental defects. The presence of lymphocytes in valvular tissue would be more difficult to explain if their identity were certain and cross sections of fibroblastic nuclei could be ruled out. Inasmuch as the cardiac inflammations have always been considered healed, the absence of scar tissue in the deformed valves is an insurmountable objection to an assumption of inflammation. The explanation offered by Niemyska,[17a] that the inflammation subsides without leaving residua, can safely be ignored in the presence of deformity.

Mönckeberg's [20] statement that an acute form of this so-called inflammation had never been encountered is of the greatest significance, notwithstanding the opinions of Cappelli [12] and de Vecchi,[26] which are based on findings of questionable significance.[48] The rarity of endocarditic processes in infants, as indicated by Ribbert,[24] further emphasizes the significance of Mönckeberg's [20] statement.

The microscopic appearance of the myocardial lesions in the fetal hearts bears a striking resemblance to that of the bland myocardial infarcts seen in adults. An interpretation of these lesions on an inflammatory basis meets with several objections: First, it would be highly unusual for so destructive an inflammation in so small an organ to remain as sharply localized to the papillary muscle as these lesions usually are, and, second, if the lesions were of inflammatory character, endarteritic vascular changes should be present in the scar tissue. Such

48. Dr. Alfred Plaut has called my attention to several of his publications (Arch. Path. **20**:582, 1935; Am. J. Path. **15**:649, 1939), which I had overlooked. These concern the presence of minute fresh fibrin thrombi on the mitral valve leaflet of one, and the tricuspid leaflet of a second, newborn infant. Leukocytes and "different cells with medium-sized simple nuclei" were diffusely scattered throughout the thrombus in the first case but not in the second case. In both instances, focal deficiencies in the lining endothelium underlying the thrombus were present, and nuclei of the valvular tissues near the lesions were swollen. No other valvular changes were noted, and no bacteria were demonstrable. Dr. Plaut suggested that such lesions might heal without major defects in the valve. It appears probable that these changes, somewhat related to those described by de Vecchi [26] and by Cappelli,[12] occurred shortly before death and their significance in, or relation to, the cardiac malformations under discussion seems doubtful.

changes, however, were not found in the present case, nor were they described in other reported cases, although mention of obliterative vascular changes in aortic atresias was made by Roberts [36] and, in the case of a 22 year old woman, by Letulle.[18] The heart described by Letulle [18] was of an age in which postnatal vascular changes were possible, and the lesions were obviously of developmental genesis since there were transposition of the great vessels, a septal defect and subvalvular stenosis.

In a consideration of infarction as an explanation of the myocardial lesions, the absence of arterioluminal, arteriosinusoidal and thebesian vessels may assume importance. Wiggers [49] expressed the belief that there is not a thebesian arterial flow of any magnitude from the ventricles to the arteries under normal dynamic conditions. It is possible, however that under the condition of patent ductus arteriosus the pressure relationships may be such that the arterioluminal and thebesian vessels may play an important role in the nutrition of the fetal myocardium. If this assumption should be correct, occlusion of these vascular channels by sclerosis of the parietal endocardium would furnish an adequate explanation of the myocardial lesions.

The diffuse endocardial fibrosis, also devoid of inflammatory residua, is best explained as simple hyperplasia of the endocardial fibroelastic tissue. Whether this is secondary to circulatory changes or to a primary developmental defect as suggested by Dissmann [30b] is easier to determine in those cases in which such endocardial sclerosis is found unassociated with valvular defects. The existence of such cases [50] appears to favor Dissmann's theory.

As a final objection to the endocarditic concept of these congenital cardiac lesions, attention is directed to the theoretic etiologic pathogen of this assumed inflammation. This pathogen, although conceded to be transmitted from mother to fetus, is characterized by its frequent anamnestic absence and variability.

SUMMARY

The occurrence of fetal endocarditis has never been established. The macroscopic and microscopic abnormalities considered criteria of fetal endocarditis have been observed also in the presence of congenital cardiac defects which, after study, have been diagnosed as noninflammatory lesions. The assumed etiologic pathogen in the reported cases of fetal endocarditis has been either anamnestically absent or variable in identity.

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49. Wiggers, C. J.: The Physiology of the Coronary Circulation, in Levy, R. L.: Diseases of the Coronary Arteries and Cardiac Pain, New York, The Macmillan Company, 1936.

50. Boger.[34a] Loh. [39]

The myocardial lesions usually seen in so-called fetal endocarditis closely resemble healed bland infarcts. Such infarctions may be related to the obliteration of arterioluminal, arteriosinusoidal and thebesian vessels by the marked parietal endocardial fibrosis.

The valvular and parietal endocardial changes seen in these cases are better explained on the basis of a developmental defect, since they show no inflammatory residua.

The case of a full term newborn boy is reported, in which stenosis of the mitral and aortic valves was present which resembled strikingly the rheumatic valvular disease of adult life. This condition was associated with hypoplasia of the root of the aorta, rudimentary development of the left ventricular cavity, the presence of a large patent ductus arteriosus and marked diffuse parietal endocardial sclerosis of the left ventricle. The genesis of these lesions is considered noninflammatory because of the lack of inflammatory residua.

GENERALIZED SARCOIDOSIS OF BOECK ACCOMPANIED WITH TUBERCULOSIS AND STREPTOCOCCIC BACTEREMIA

A CLINICOPATHOLOGIC STUDY WITH AUTOPSY AND ANIMAL INOCULATIONS

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The possibility that Boeck's sarcoid is a noncaseating form of tuberculosis has excited a controversy which remains unsettled. Pinner [1] recently reviewed the arguments for and against this theory. Schaumann [2] followed patients with sarcoid for periods up to eighteen years and in some found at autopsy typical caseous tuberculosis, but in these the noncaseating sarcoid lesions had disappeared. He recovered tubercle bacilli from animals inoculated with the caseous tissue, but not from those inoculated with the noncaseous lesions. He was convinced that the sarcoid lesions were due to infection by a dissociated or an atypical form of the tubercle bacillus in persons who responded in some unusual manner. Interest in the study of the disease in this country was stimulated by Longcope and Pierson.[3] One of us has recently completed chemical [4] and etiologic [5] studies in 11 cases. No proof of tuberculous infection was discovered in the three last-named investigations. The opportunity of studying at autopsy a patient with sarcoid who died of an intercurrent acute process and who had typical lesions of both sarcoid and tuberculosis in the same organs is rare. Attempts to recover tubercle bacilli by the inoculation of animals with material obtained at autopsy have been made very infrequently. We have made such an attempt and have recovered tubercle bacilli from one lesion only.

From the Departments of Pathology and Medicine, Duke University School of Medicine and Duke Hospital.

1. Pinner, M.: Am. Rev. Tuberc. **37**:690, 1938.
2. Schaumann, J.: Brit. J. Dermat. **48**:399, 1936.
3. Longcope, W. T., and Pierson, J. W.: Bull. Johns Hopkins Hosp. **60**: 223, 1937.
4. Harrell, G. T., and Fisher, S.: J. Clin. Investigation **18**:687, 1939.
5. Harrell, G. T.: Arch. Int. Med. **65**:1003, 1940.

LITERATURE

Few cases of sarcoid in which autopsies were done had been studied adequately before death. Bernstein, Konzlemann and Sidlick [6] reported a case in which the condition was recognized clinically and subsequently examined post mortem. Mylius and Schürmann presented 2 cases, but in 1 there was associated tuberculosis.[7] Schaumann [2] recorded the pathologic observations in 4 persons with the disease who came to autopsy. Three of these had had thorough clinical investigation, and the true nature of the condition was recognized before death. Two of these patients later died of active tuberculosis, the sarcoid lesions having previously undergone spontaneous regression. The fourth patient died of intercurrent disease (influenza), and sarcoid was not suspected before autopsy. Nickerson [8] recorded the pathologic observations on autopsy material in 6 cases. The presence of sarcoid had not been suspected before death, and clinical studies of the condition had not been carried out. Spencer and Warren [9] presented a thoroughly studied case, which was followed for two years; the diagnosis had been made ante mortem and was confirmed at autopsy. Cotter [10] recently reported the case of a patient who died of progressive myocardial failure due to the presence of proliferating sarcoid lesions in the myocardium; the true nature of the condition was not recognized before autopsy was performed. Danbolt and Hval made the postmortem examination in one of Boeck's original cases, in which the illness terminated with a cancer twenty-nine years after sarcoid was first diagnosed, and found the lesions still present.[11] Pinner mentioned 5 other cases of sarcoid lesions and added 1 case of his own, but 3 of the patients had active tuberculosis.[1] It is doubtful that all of these were actually instances of sarcoid.

Altogether, 22 cases in which autopsies have been made and sarcoid diagnosed have been found recorded in the literature. In 8 of these there were associated caseous tuberculous lesions, in which acid-fast organisms were demonstrated.[12] Caseous lesions were described in other cases, but no acid-fast organisms were identified. Reports of animal inoculations from autopsy material were made in only 6 of the 22 cases. Guinea pig inoculations were positive for tuberculosis in Schaumann's case 3, "a typical case of lymphogranulomatosis benigna ultimately complicated with tuberculosis," and in Schlagenhaufer's case, cited by Kyrle.[13] Guinea pig inoculations produced no results in Schaumann's [2] cases 2 and 4 and in those of Spencer and Warren [9] and Danbolt and Hval.[11]

REPORT OF A CASE

A 19 year old Negro, unmarried, was admitted to Duke Hospital, Nov. 12, 1939, complaining of enlargement of lymph nodes, which had been present for four years.

6. Bernstein, M.; Konzlemann, F. W., and Sidlick, D. M.: Arch. Int. Med. **44**:721, 1929.
7. Mylius, K., and Schürmann, P.: Beitr. z. Klin. d. Tuberk. **73**:166, 1929.
8. Nickerson, D. A.: Arch. Path. **24**:19, 1937.
9. Spencer, J., and Warren, S.: Arch. Int. Med. **62**:285, 1938.
10. Cotter, E. F.: Arch. Int. Med. **64**:286, 1939.
11. Danbolt, N., and Hval, E.: Acta dermat.-venereol. **17**:477, 1936.
12. Pinner.[1] Schaumann.[2] Mylius and Schürmann.[7]
13. Kyrle, J.: Arch. f. Dermat. u. Syph. **119**:117, 1914. Schaumann.[2]

An uncle had had tuberculosis. The patient had never been robust and had frequent upper respiratory infections. In 1935, gradual, progressive, painless enlargement of the inguinal lymph nodes began, followed by enlargement of the cervical, submental, axillary, epitrochlear, postauricular and occipital groups. In October

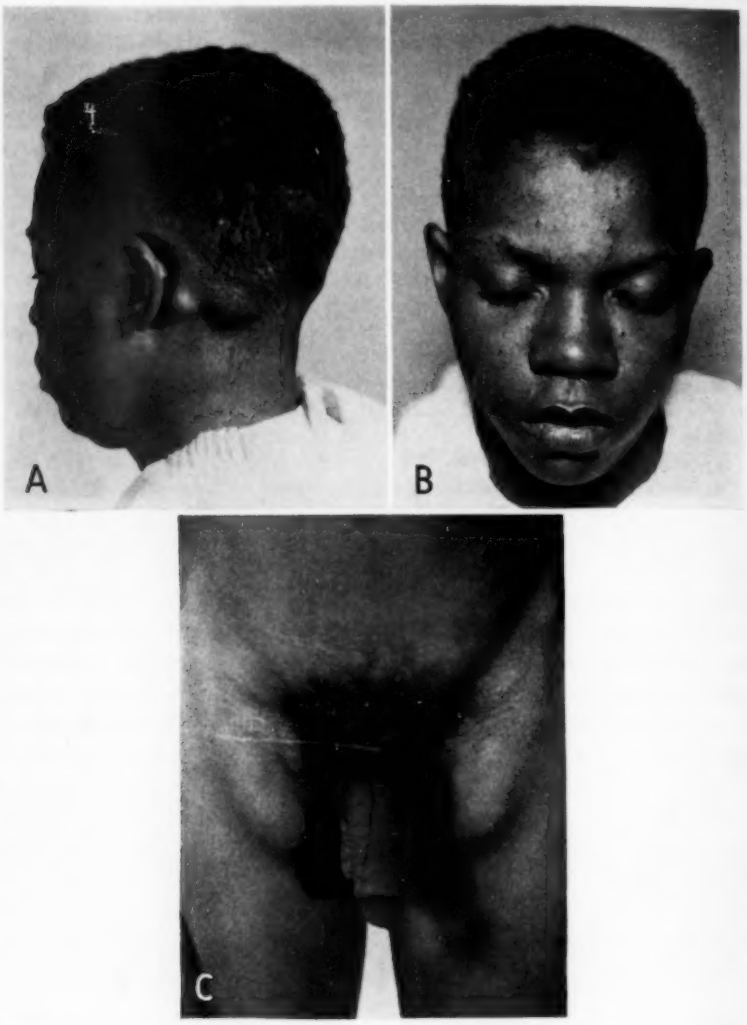

Fig. 1.—*A*, enlargement of preauricular and postauricular, posterior cervical and submandibular lymph nodes; *B*, cutaneous lesions of the face; *C*, femoral and inguinal lymph nodes.

1937, cough productive of mucopurulent sputum developed; he lost 6 pounds (2.5 Kg.) in weight and began to have pain in the chest and afternoon fever. Small circular dry skin lesions, persistent sore throat and hoarseness developed.

Pain and swelling of the terminal phalanges of the fingers and toes lasted for two months. He was treated in the North Carolina (State) Sanatorium from August 15, 1938 until June 22, 1939 with rest in bed and with sulfanilamide and potassium iodide up to 16.2 Gm. daily, without improvement. Roentgen examination showed fine, snowflake-like densities evenly distributed throughout both lungs, most marked in the right apex (fig. 2 *A*). Dr. P. P. McCain, Sanatorium, N. C., lent us the roentgen films and gave us a summary of the findings. Cultures for fungi and inoculations of the sputum in guinea pigs gave negative results. The skin reaction to 1 mg. of old tuberculin was negative, but by February 1939 it was 2 plus to a dilution of 1:10,000.

On admission the temperature was 38 C. (100.4 F.), the pulse rate 100, the respirations 20 and the blood pressure 110 systolic and 60 diastolic. The patient was thin and poorly developed. On the extensor surfaces of the arms and legs and on the buttocks were numerous discrete rounded painless papular lesions, measuring 1 to 4 mm. in size, with crusted centers and surrounding depigmentation. On the forehead, eyelids and upper lip were 1 to 2 mm. translucent soft round nodular lesions (fig. 1 *B*). All the lymph nodes were enlarged, including those of the subcutaneous tissue of the shoulders; the largest, 4 by 6 cm. in size, were in the inguinal region (fig. 1 *A* and *C*). The tonsils were large and red. A few fine dry inspiratory rales were heard over the midlung fields posteriorly. The heart was not enlarged, and no murmurs were heard. No abdominal organs were palpable. No swelling of the fingers or toes was present.

The hemoglobin content was 12.6 Gm. per hundred cubic centimeters; white blood cells, 7,300, with 66 per cent segmented polymorphonuclears, 1 per cent stab forms, 3 per cent eosinophils, 1 per cent basophils, 18 per cent small lymphocytes, 9 per cent large lymphocytes and 2 per cent monocytes. The sedimentation rate of the erythrocytes was 18 mm. per hour. The Wassermann and Kahn tests were negative. The blood proteins totaled 7.1 Gm. per hundred cubic centimeters, with 4 Gm. globulin; calcium was 10.1 mg., phosphorus 3.8 mg., phosphatase 6.5 Bodansky units and cholesterol 194 mg. The bromsulphalein test was negative. Four grams of ascorbic acid was required to saturate the body. Roentgen films of the chest showed no change over those of the previous year, but numerous punched-out areas were found in the phalanges of the fingers and toes (fig. 2 *B*). No Bence Jones protein was present in the urine. The skin reaction with old tuberculin in the dilution of 1:100 was 2 plus. Each of ten consecutive positive blood cultures showed less than one colony of alpha hemolytic streptococci per cubic centimeter. Two electrocardiograms were normal.

The temperature remained below 38 C., with a pulse rate of 100 to 120. On the ninth hospital day tonsillectomy was done, with the patient under nitrogen monoxide–oxygen–ether anesthesia. The temperature rose sharply to 40.5 C. (104.9 F.), with a pulse rate of 140. On the fourth postoperative day the temperature fell, and the patient appeared stronger, but he suddenly became comatose, with a blood pressure of 90 systolic and 60 diastolic, a pulse rate of 180, contracted pupils, cyanosis and moist rales throughout both lungs; he died three hours later.

Autopsy.—The anatomic diagnosis was: Boeck's sarcoid, involving the skin, lymph nodes (generalized), lungs, liver, spleen, kidneys, peritoneal surfaces of the diaphragm and stomach, tonsils, epiglottis, bone marrow of vertebrae, ribs and phalanges of toes, and phalangeal synovial membrane.

Autopsy was performed eight hours after death. The cutaneous lesions described clinically were noticeably smaller. Numerous fine fibrous bands extended from the visceral peritoneal surface of the small intestine to the anterior and lateral

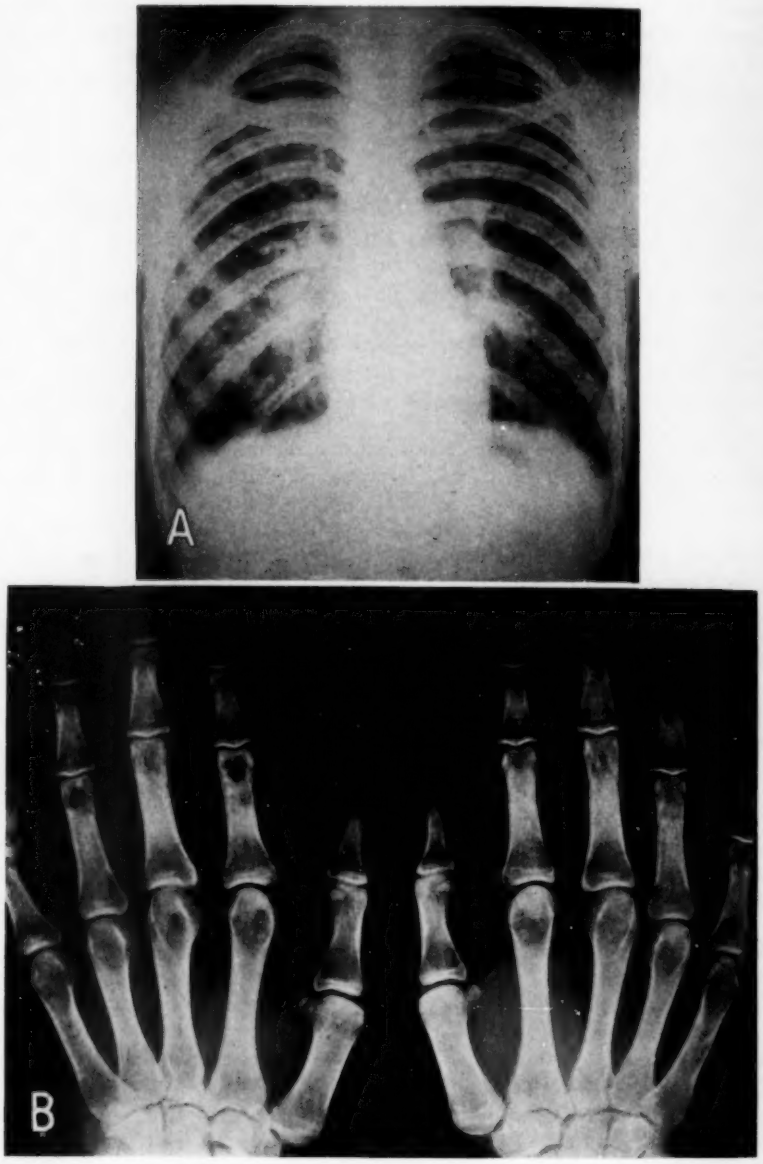

Fig. 2.—*A*, miliary nodules in the lungs, sparing the apex of the left lung and the bases of both lungs. *B*, cystlike punched-out areas in the phalanges of the hands.

parietal peritoneum. Each pleural cavity contained 100 cc. of clear straw-colored fluid, and thin fibrous adhesions were present at the left apex.

Lungs: The left lung weighed 1,130 Gm. and the right 1,080 Gm. The subpleural surfaces of both were studded with irregular, slightly elevated, pale green plaques, measuring 1 to 3 mm. in size, which were slightly firmer than the surrounding parenchyma (fig. 3 *B*). On section, the cut surfaces, which exuded an abundance of frothy fluid, were firm, indurated, pinkish gray and studded with firm, grayish white, shotlike nodules, measuring 1 to 3 mm. in diameter (fig. 3 *A*).

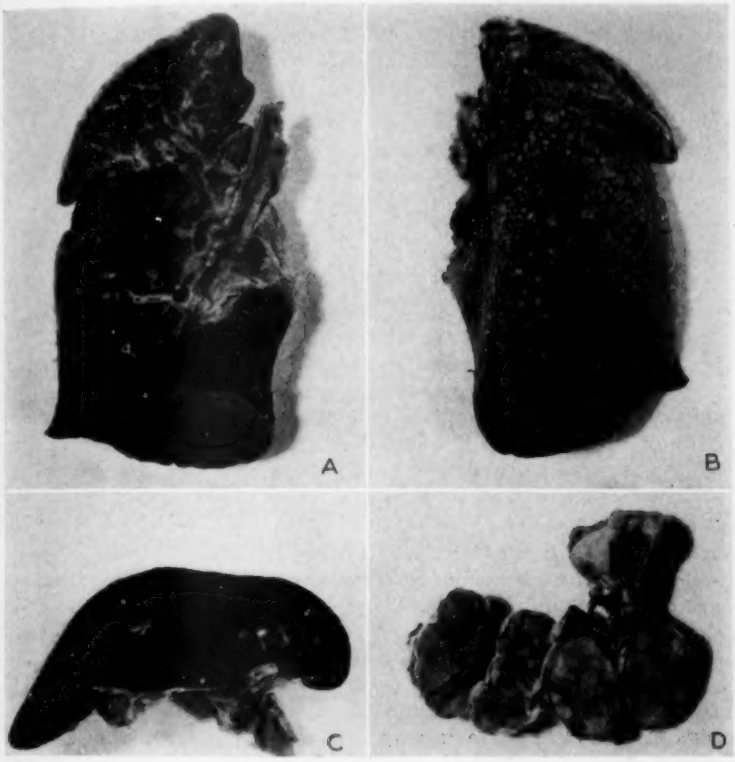

Fig. 3.—*A*, right lung, showing fibrosis, thickening and ulceration of bronchi, and enlargement of hilar lymph nodes; *B*, right lung, showing tubercle-like lesions without adhesions on the pleural surface; *C*, spleen showing fibrosis and tubercles; *D*, lymph nodes showing fibrosis without necrosis or thickening of the capsule.

The interlobar septums and the walls of the small and large bronchi showed extensive scarlike thickening, which decreased the size of the lumens of the bronchi. In some places, the bronchial mucosa was ulcerated.

Liver: The liver weighed 1,510 Gm. The capsule was thickened and densely adherent to the adjacent diaphragm, and presented on the surface scattered, slightly elevated, firm, grayish white nodules. The cut surface showed numerous nodules, measuring 1 to 2 mm. in diameter.

Spleen: The spleen weighed 200 Gm. It was firmly adherent to the diaphragm, the colon and the parietal peritoneum. In the capsule were many slightly elevated, grayish white plaques, measuring 1 to 3 mm. in diameter. The cut surface showed numerous rounded firm nodules, measuring 1 to 3 mm. in diameter (fig. 3 *C*).

Lymph Nodes: The pretracheal, bronchopulmonary, mesenteric, gastric, portal, para-aortic, iliac and retroperitoneal nodes were all enlarged, discrete and rubbery. They measured from 1 to 6 cm. in diameter. The cut surfaces bulged slightly and showed numerous concentric, grayish white confluent masses of tubercles, giving a lobulated appearance (fig. 3 *D*).

The contents of the cranial cavity, including an eye, showed no abnormality.

Microscopic Observations.—The sarcoid lesion, in all the involved organs, consisted of a collection of discrete and confluent tubercles. These were of two general types:

1. Epithelioid tubercles, both discrete and confluent, consisting of collections of epithelioid cells, which centrally were distributed irregularly but peripherally were concentrically arranged (fig. 4 *A* and *C*). The elliptic macrophages were well defined, and only a small amount of collagen surrounded the tubercles. Large giant cells, containing from 15 to 50 nuclei, were present. About the periphery of the tubercles were dense collections of lymphocytes.
2. Other tubercles partially or wholly made up of homogeneous eosinophilic material, which was identified as fibrous tissue when stained with Mallory's collagen stain; congo red stains for amyloid were negative (fig. 4 *D*). This replacement process appeared first to involve the peripheral zone of the tubercle, then to progress centrally, involving the entire tubercle. Many of the tubercles were acellular, while others showed a poorly defined central zone of irregularly arranged epithelioid cells. Still others showed numerous elongated, spindle-shaped cells having the appearance of fibroblasts. Giant cells were not seen. Peripheral collections of lymphocytes were rare or absent.

A third type of tubercle, found only in the liver and spleen, was composed of a central caseous area surrounded by a concentric zone of well defined epithelioid cells and an outer dense zone of lymphocytes (fig. 4 *B*).

Polymorphonuclear cells and eosinophils were conspicuously absent in each type of lesion. No acid-fast bacilli were stained in any tissue by the Ziehl-Neelsen method.

Lungs: Discrete and confluent collections of noncaseating epithelioid and fibroepithelioid tubercles were disseminated abundantly throughout both lungs. The denser areas of epithelioid proliferation were present where normally a greater condensation of lymphoid tissue and a larger number of lymphatic channels existed. The larger collections, made up of dense fibrous connective tissue and fibroepithelioid tubercles, were found around the hilus. In the peripheral pulmonary parenchyma the wall of the interalveolar septums were thickened, with partial or complete obliteration of the air space; the interlobular septums were transformed into dense bands of epithelioid, fibroepithelioid tubercles and fibrous tissue. The walls of many blood vessels were thickened, and occasionally the lumen was almost completely obliterated by a hyaline mass. Dense collections of fibroepithelioid tubercles and collagen surrounded the bronchi, causing mural thickening and partial or complete compression of the lumens. The pleura was irregularly thickened by dense aggregations of fibroepithelioid tubercles, fibrous tissue and many dilated blood vessels.

Liver: Occasional caseating tubercles were present in the hepatic parenchyma. The characteristic epithelioid and fibroepithelioid tubercles were present in the interacinar connective tissue, most frequently in the portal areas. The parenchymal cells showed moderate atrophy and in many areas fatty change.

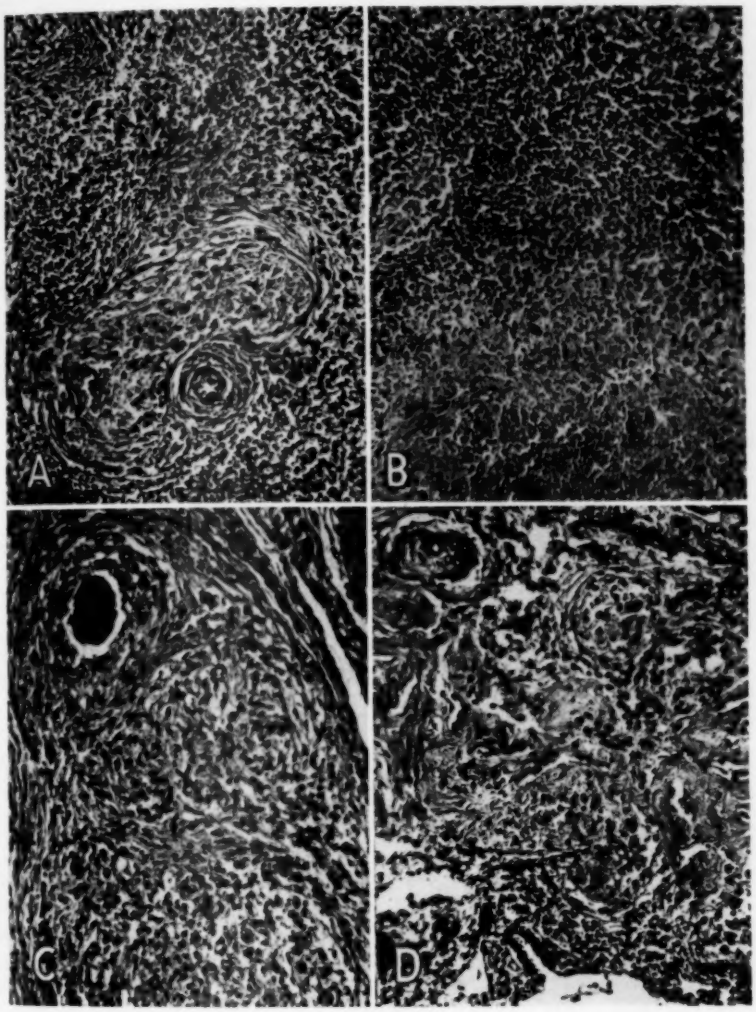

Fig. 4.—*A*, noncaseous lesion in the spleen; *B*, edge of caseous lesion in the spleen; *C*, proliferative tubercle-like lesion with giant cell from peritoneal surface of the diaphragm; *D*, older hyalinized lesion in the lung. × 83.8.

Spleen: Epithelioid and fibroepithelioid tubercles were most abundant in the splenic pulp, but were present in the malpighian corpuscles. One large tubercle showed a central area of caseation and a surrounding zone of epithelioid cells. The plaques in the capsule were collections of fibrous and fibroepithelioid nodules.

Fig. 5.—The left fourth toe, showing complete replacement by sarcoid tissue of the marrow cavity in the middle phalanx at the site of a cystlike area in the roentgenogram. Note the protrusion of tubercles, proliferating on the synovial membrane, into the interdigital spaces. × 9.6.

Kidneys: One aggregation of confluent epithelioid tubercles was seen in a pyramid of the left kidney. A moderate number of lymphocytes and a few giant cells were present.

Lymph Nodes: All lymph nodes were made up almost entirely of confluent aggregations of epithelioid and fibroepithelioid tubercles. Giant cells were seen only occasionally.

Animal Inoculations.—Animals were inoculated with material from an inguinal lymph node and from the tonsils during life, with blood from a cardiac puncture and with spinal fluid from a cisternal puncture immediately after death, and with tissue from the upper lobe of the right lung and from a lymph node near the hilus of the liver at autopsy. The inoculations were made in 10 guinea pigs, 6 mice and 1 rabbit. The mice all died within two days, from peritonitis and septicemia; an organism similar to that recovered from the blood during life was cultured from all. Three guinea pigs died within the first week, and another within six weeks, as a result of infection with the streptococcus; still another died of intercurrent pneumonia after three and a half months. Two guinea pigs were put to death after four months, and 2 more and the rabbit after five months; all had been negative on skin tests to 5 mg. of old tuberculin and to 0.1 cc. of 1:1,000 brucellergin. In none of these animals which survived the first week was gross or microscopic evidence of tuberculosis found. One guinea pig inoculated with material from the abdominal lymph node died after two and a half months with generalized caseous tuberculosis, and acid-fast organisms were readily stained in the lungs; the mate, which had a positive tuberculin test, died after four months with gross caseous lesions in the lungs only.

Cultures made directly from the material removed during life and at autopsy yielded only varied gram-positive cocci on aerobic and anaerobic blood agar, broth and Sabouraud mediums. On appropriate mediums no acid-fast organisms were grown from the inguinal lymph node.

A tissue culture made of the inguinal node by Dr. Duncan C. Hetherington, of the department of anatomy, produced some very long, slightly granular fibroblasts, which were thought not to be abnormal.

COMMENT

The theory that sarcoid is a form of noncaseating tuberculosis is accepted widely. The failure of numerous investigators to recover tubercle bacilli from the lesions by inoculation of animals or to stain the bacilli in tissue has been explained on the ground that the organisms are in a filterable form or have dissociated into atypical partially acid-fast or nonacid-fast forms. The cultural, serologic and sensitivity researches of Schaumann,[2] Kyrle [14] and Mellon and Beinhauer [15] offer some support for this view. The absence of positive skin reactions to protein fractions of tubercle bacilli has been explained by an anergic state of the host [1] or by the presence in the blood of anticutins which neutralize the injected tuberculoprotein.[16] An anergic state or the

14. Kyrle, J.: Arch. f. Dermat. u. Syph. **131**:33, 1921.

15. Mellon, R. R., and Beinhauer, L. G.: Arch. Dermat. & Syph. **36**:515, 1937.

16. Sulzberger, M. B., and Wise, F.: M. Clin. North America **14**:1555, 1931.

presence of anticutins, it appears to us, should not prevent the recovery of tubercle bacilli from the lesions by animal inoculation. In contrast to the view just stated, other investigators feel that the tubercle bacillus is not the proved etiologic agent and that the cause is still unknown.[17]

The presence of both caseating and noncaseating lesions in the liver and spleen, in this case, casts doubt on the theory that there is a change in the reactivity of the tissue (anergy) to the usual form of tubercle bacillus, if that is the etiologic agent. We found no intermediate stages between the caseous and the noncaseous lesions. It is unfortunate that microscopic sections were not made from a portion of the node which yielded acid-fast organisms on animal inoculation, for no caseation was found in the sections of node, tonsils or lung which yielded no acid-fast organisms.

It is possible that this patient had two diseases: a minimal tuberculous infection and sarcoid. The streptococcic bacteremia was thought to be incidental and not significant in the genesis of the tubercle-like lesions. This point of view could explain the past history of a transient positive reaction to tuberculin and the recovery of tubercle bacilli from a node draining the liver, in which rare caseous lesions were found.

SUMMARY

A patient with generalized Boeck's sarcoid who had been studied clinically for two years died suddenly, and an autopsy was performed. The lesions of sarcoid were found in nearly all the organs, including a joint capsule and the epiglottis, where they rarely are reported. Several caseous lesions were found in the liver and spleen. The 22 recorded autopsies are reviewed briefly.

Tubercle bacilli were recovered in guinea pigs inoculated with material from a lymph node from the hilus of the liver. None were recovered from animals inoculated with femoral node, tonsil, lung, spinal fluid and blood.

The patient may have had two diseases: localized tuberculosis, from which organisms were recovered, and sarcoid, the etiologic agent of which remains unidentified. A simultaneous streptococcic bacteremia was thought not to be significant.

17. Longcope and Pierson.[3] Harrell.[5]

VISCERAL LESIONS FOLLOWING SINGLE SUBCUTANEOUS INJECTION OF BENZPYRENE IN MICE

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In studies on tumors produced experimentally with benzpyrene or other carcinogenic substances, certain gross and microscopic alterations have been described. Histologic investigations were made of tissues at or near the site of application of the carcinogenic substances. These substances were applied by repeated painting, by subcutaneous injection or by insertion of pellets (Haagensen and Krehbiel;[1] Dunning, Curtis and Bullock;[2] Wolbach;[3] Rondoni;[4] Orr;[5] Brock, Druckrey and Hamperl;[6] Twort and Twort,[7] and others). The local findings consisted chiefly of fatty degeneration and necrosis of the tissue involved and reactive formation of granulation tissue with irregular proliferation and hyperplasia of the connective tissue. There seems to be general agreement that the tumor frequently forms at the borderline between the necrotic and the granulation tissue.

Other investigators, in addition to describing the tissue changes at the site of application of the carcinogenic substances, also noted alterations within internal organs. Lesions were described not only during the early period, before tumor formation, but also in later stages, four or five months after the application, both in animals with and in those without tumor formation. Picard and Laduron[8] observed splenic atrophy,

From the Laboratories of the Mount Sinai Hospital.

Read before the American Association of Pathologists and Bacteriologists, Pittsburgh, March 21, 1940.

1. Haagensen, C. D., and Krehbiel, O. F.: Am. J. Cancer **26**:368, 1936.
2. Dunning, W. F.; Curtis, M. R., and Bullock, F. D.: Am. J. Cancer **28**: 681, 1936.
3. Wolbach, S. B.: Am. J. Path. **13**:662, 1937.
4. Rondoni, P.: Ztschr. f. Krebsforsch. **47**:59, 1937.
5. Orr, J. W.: J. Path. & Bact. **46**:495, 1938.
6. Brock, N.; Druckrey, H., and Hamperl, H.: Arch. f. exper. Path. u. Pharmakol. **189**:709, 1938; Arch. f. klin. Chir. **194**:250, 1939.
7. Twort, J. M., and Twort, C. C.: J. Path. & Bact. **35**:219, 1932; Am. J. Cancer **35**:80, 1939.
8. Picard, E., and Laduron, H.: Compt. rend. Soc. de biol. **115**:1739, 1934; **117**:838, 1934.

depletion of lymphatic tissue in lymph nodes and in the thymus and the frequent presence of pleural effusion. Burrows and Cook,[9] Parsons,[10] Lewis[11] and Leuchtenberger[12] reported leukemoid reactions with splenohepatomegaly or myeloid metaplasia and infiltration in different organs, especially in the spleen and liver and occasionally in lymph nodes. Other visceral lesions, such as fatty degeneration, dissociation of liver cell cords, periportal proliferation and cirrhosis (Polson;[13] Claude;[14] Wolbach;[3] Goerner and Goerner[15]), have been reported. However, the findings have not been constant; for instance, Polson emphasized negative results after treatment of rabbits with 3,4-benzpyrene.

Similar alterations have been seen by us in mice that received a single subcutaneous injection of 1,2-benzpyrene. In addition, we encountered a number of unusual visceral lesions, and these are the subject of the present report.

MATERIAL AND METHODS

White female Rockland mice about 2 months of age were used in all experiments. They were kept on the standard "Rockland" diet. Injections of benzpyrene were made in 869 mice. Of these, 534 were given a single subcutaneous injection of 0.5 cc. of a 0.4 per cent solution of 1,2-benzpyrene in lard, and 335 mice received 0.5 cc. of a 0.03 per cent colloidal solution of benzpyrene. The mice survived from one to sixteen months; 65 are still alive. It might be mentioned that to date 232 tumors (sarcomas) have developed in the mice; 208 resulted from the injection of benzpyrene in lard and only 24 from the injection of colloidal benzpyrene. The difference in the tumor development may be explained by the fact that only one tenth of the amount of benzpyrene in the solution in lard was injected in the colloidal solution. As the chart shows, most of the tumors (93) developed in the fourth month after the injection of the benzpyrene in lard.

TABLE 1.—*Tumor Distribution in Successive Months*

Solution Injected	Total Number of Tumors in Percentage	Distribution of Tumor Occurrences in Months											
		1	2	3	4	5	6	7	8	9	10	11	12
Benzpyrene in lard.....	38.9	0	2	10	93	49	24	12	5	4	4	4	1
Colloidal benzpyrene..	7.2	0	0	0	2	8	7	2	1	1	1	2	0

It is not our purpose, however, to report on tumor formation.

9. Burrows, H., and Cook, J. W.: Am. J. Cancer **27**:267, 1936.

10. Parsons, L. D.: J. Path. & Bact. **40**:15, 1935; **43**:1, 1936.

11. Lewis, M. R.: Am. J. Cancer **34**:431, 1938.

12. Leuchtenberger, C.: Separation and Passage of a Leukemoid State Observed in Mice Bearing Benzpyrene Sarcomas, read at the Third International Cancer Congress, Atlantic City, N. J., Sept. 11-15, 1939. A report of this congress was not published (owing to war). An excerpt of the paper was printed in the program of the Third International Congress, 1939, pp. 163-164.

13. Polson, C.: J. Path. & Bact. **42**:537, 1936.

14. Claude, A.: Am. J. Cancer **31**:100, 1937.

15. Goerner, A., and Goerner, M. M.: Am. J. Cancer **37**:518, 1939.

CLINICAL OBSERVATIONS

About six weeks after injection of the benzpyrene, many mice became ill, as evidenced by ruffling of the fur and loss of hair. Other significant signs of progressive decline included arrest of development, loss of weight, dehydration, cachexia, more or less generalized edema, loss of muscle tone and partial or complete paralysis. Occasional animals showed periorbital edema with suppurative blepharitis. Control animals of the same stock and age were unaffected. The peak of the illness appeared at about the fifth to the seventh month after the injection; many of the animals died at this time.

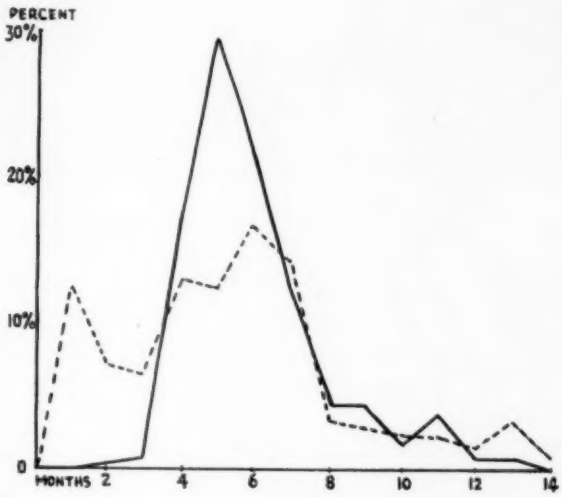

Fig. 1.—Mortality curve, in percentage, of tumor-bearing mice (solid line) and of mice in which no tumors developed (broken line). The numerals at the bottom refer to months after injection of benzpyrene.

Figure 1 compares the percentage of deaths among tumor-bearing mice with that among the mice without tumors over a period of fourteen months. While the mortality of the tumor-bearing animals reaches a peak between the fifth and the sixth month, the mortality in the mice in which no tumors developed is more scattered. Of 869 mice given injections, 572 died without tumors. These facts seem to indicate that there must be some cause of death other than the growing neoplasm in this group.

POSTMORTEM OBSERVATIONS

Gross Examination.—The gross findings in these mice frequently corresponded to the clinical observations. Some of the animals showed not only edema of the subcutaneous tissue and muscles but also serous

effusions into the abdominal, pleural and pericardial cavities and edema of the intestine, mesentery and region around the pancreas. The heart was often dilated and the myocardium grayish white. Other animals

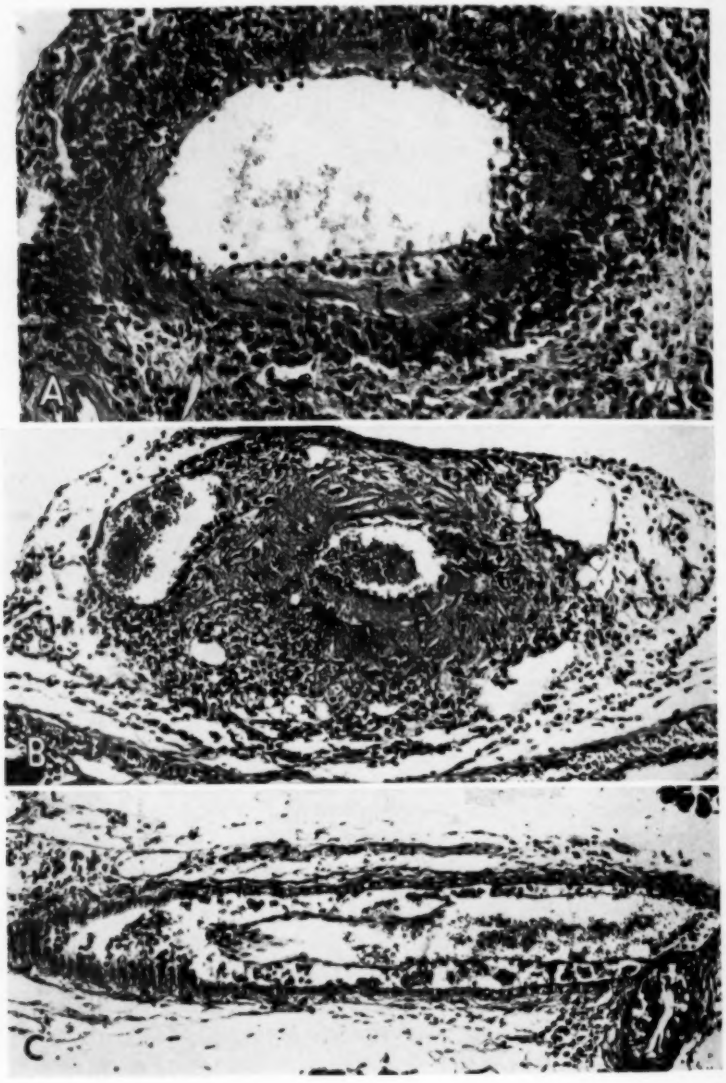

Figure 2

(See legend on opposite page)

presented marked splenohepatomegaly and an uneven surface of the liver. The gallbladder was either edematous or small and fibrotic; similarly the urinary bladder.

Of the mice given injections, 340 were examined histologically. Of these, 99 were tumor-bearing mice and 241 had no tumors. On histologic examination a variety of visceral lesions was encountered.

We examined microscopically chiefly the heart and blood vessels, the liver and gallbladder, the kidney and urinary bladder, the lungs, the stomach and intestines, and the fat and connective tissue of the mesentery, of the areas around the spleen and pancreas and of the areas around the lymph nodes. Occasionally, the skin and muscle were studied.

Microscopic Examination.—The most striking findings were the vascular lesions in many organs. While about 20 per cent of the animals showed a more or less extensive perivascular infiltration without involvement of the vessel wall, 31 per cent presented vessel changes of a severe character in at least two or more organs. These were in the nature of necrotizing panarteritis as seen in periarteritis nodosa (figs. 2 *A* and *B*). Another significant vascular lesion, seen especially in the early stages after the injection of benzpyrene, was generalized perivascular and mural edema, which sometimes resulted in complete dissolution of the layers of the vessel wall (fig. 2 *C*).

Striking lesions were seen in the heart. More than one third of the mice presented diffuse myocarditis and an inflammatory reaction around or in the wall of the coronary artery or of the great vessels at the base of the heart (figs. 3 *A* and *B*).

Hepatic alterations were observed frequently. These consisted of focal lymphocytic and myeloid infiltrations in the periportal fields or

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EXPLANATION OF FIGURE 2

A, cross section of a coronary artery after one subcutaneous injection of colloidal benzpyrene (mouse 291, between six and seven months after injection). There are intimal proliferation, homogenization of the media, with bizarre nuclei, evidence of fibrinoid necrosis and massive perivascular infiltration by mononuclear elements. Many of these show necrobiosis.

B, the subserosa of the intestine after one subcutaneous injection of colloidal benzpyrene (mouse 183, between four and five months after injection). There is an extensively damaged vessel, showing necrotizing arteritis. This lesion is quite representative, being seen in the urinary bladder, the fat and the connective tissue, as well as in the gastrointestinal tract, the muscle tissue, the splenic hilus and the pancreas, and in areas around lymph nodes in many of our cases.

C, an artery of the spleen-pancreas region after one subcutaneous injection of colloidal benzpyrene (mouse 183). A fresh edematous lesion of a large vessel may be seen. The endothelium is lifted up. Between the endothelium and the media are many mononuclear and polymorphonuclear leukocytes. The media appears partly swollen and loosened, and the cells show some impairment of staining power. The adventitia is also loosened, and cell accumulations are visible.

areas of parenchymal necrosis and edema. A striking hepatic change was a diffuse inflammatory mesenchymal reaction with proliferation of the reticuloendothelial cells (figs. 4 *A* and *B*). Especially in later months, a peculiar kind of fat cell formation was observed inside of the broadened portal fields (fig. 4 *C*).

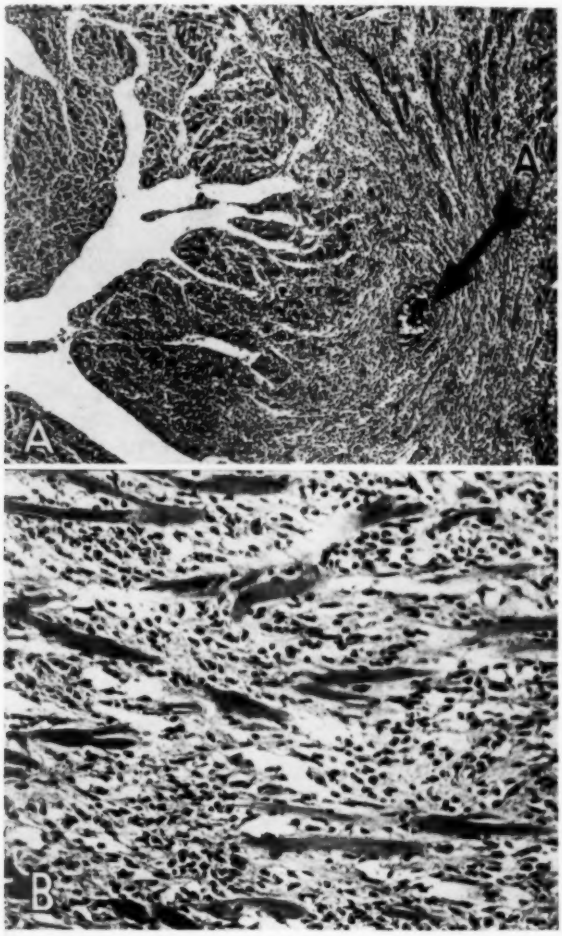

Fig. 3.—*A*, low power view of heart five months after one subcutaneous injection of colloidal benzpyrene (mouse 207). There is severe diffuse myocarditis. The muscle fibers are replaced by granulation tissue showing cellular infiltration, chiefly mononuclear elements intermingled with polymorphonuclear cells in foci. A large branch (*A*) of a coronary artery participates in the process. *B*, a high power view of the same heart. Diffuse cellular infiltration separates adjacent muscle bundles. There is marked destruction of muscle fibers. Large mononuclear histocytic cells and fibroblasts are dispersed irregularly among collagen fibers.

OBSERVATIONS IN NORMAL MICE

From the thorough studies of Wilens and Sproul [16] on spontaneous cardiovascular lesions in rats, it is known that in rats, mainly in the third year, inflammatory and degenerative cardiac and vascular lesions may occur spontaneously. Similar observations were made by Gross and Loewe [17] in mice. These authors found auriculitis and slight valvular and pericardial inflammatory and degenerative changes in 30 per cent of untreated white laboratory mice.

In view of the fact that histologic lesions may occur quite frequently in different organs of normal mice, we considered it necessary to study the frequency and degree of such lesions in normal Rockland mice of the same age (2 to 14 months) and sex as our experimental animals.

In these control animals we not rarely found perivascular cell infiltrations in several organs (fig. 5 *A*). Mild focal myocarditis (fig. 5 *B*) was also observed not infrequently, limited mostly to the auricle. However, widespread lesions, especially in the ventricle, were not encountered, and generalized necrotizing arteritis was never observed in untreated mice.

It seemed possible to classify the alterations observed in the experimental and control animals according to the degree of extension and severity as slight, moderate or marked. For instance, when we encountered a vascular lesion, we called the lesion slight if only small lymphoid perivascular cell accumulations, with occasional mild focal degeneration of the vessel wall, were seen. When dealing with more extensive perivascular infiltration or periarteritic or panarteritic necrotizing processes, we classified the lesions as moderate (fig. 5 *A*) or marked (fig. 2 *A* and *B*).

In the organs themselves, as in the heart or liver, we also graded the lesions according to the severity and extent of the alteration. For instance, a slight lesion in the heart was characterized by a lymphocytic reaction limited to the auricular wall or to the valves. More extensive lesions involving the ventricles were called moderate (fig. 5 *B*). Lesions were classified as marked not only because of their intensity (fig. 3 *A*) but also because of the difference in the type of reaction, a type characterized by widespread proliferation of histiocytes and fibroblasts associated with lymphocytic and polymorphonuclear leukocytic infiltration (fig. 3 *B*).

The lesions observed in the controls are shown in table 2. As may be seen from this table, only 3 mice had lesions in the heart and 15 mice lesions of moderate type in the liver. There were no marked alterations in these organs. Marked vascular lesions were never encoun-

16. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:177 and 201, 1938.

17. Gross, L., and Loewe, L.: Personal communication to the authors. Loewe, L., and Lenke, S. E.: J. Exper. Med. **71**:89, 1940.

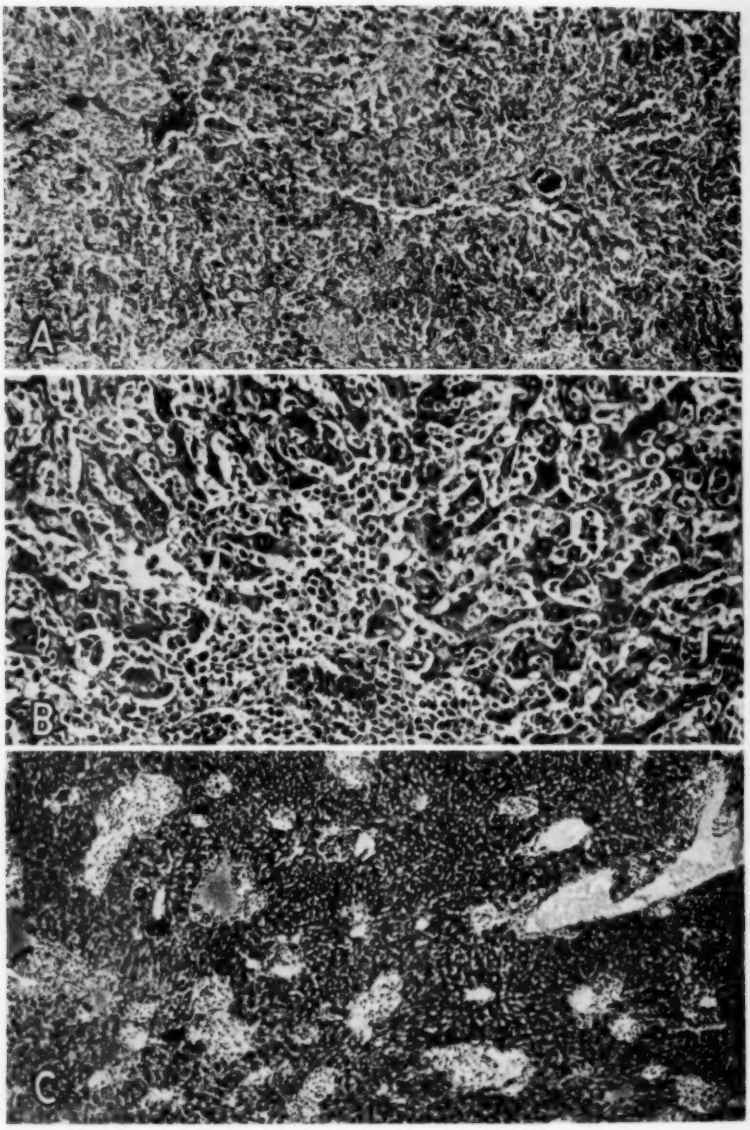

Figure 4

(See legend on opposite page)

tered in these controls. The moderate changes in the liver consisted either of more extensive perivascular infiltration in the portal fields or of some necrosis with surrounding leukocytic cell accumulations in the parenchyma. The cell infiltrations around the vessels were mostly lymphocytic or lymphoid, a fact which alone makes them differ from the cellular reactions seen after benzpyrene injections. After treatment with benzpyrene, myeloid metaplasia is predominant. A striking exception to the slight or rarely found moderate lesions in the different organs of normal mice was noted in the kidneys and to a lesser degree

TABLE 2.—*Incidence and Distribution of Histologic Lesions in One Hundred Untreated Normal Female Rockland Mice Two to Fourteen Months Old*

Organ	Number of Organs Examined	Slight	Moderate	Marked
Lungs	100	33	10	0
Heart	100	31	3	0
Liver	100	51	15	0
Gallbladder	100	9	1	0
Fat and connective tissue of mesentery and of region around spleen and pancreas	100	16	3	1
Stomach and intestines	100	31	7	0
Kidney	100	39	36	3
Urinary bladder	85	32	6	0

in the lungs of these mice. In the kidneys not only extensive perivascular infiltration, mostly lymphoid, but lesions of the vessel walls were seen. These consisted mostly of degenerative (hyaline) changes and thickening of the entire wall, with narrowing of the lumen. In a

EXPLANATION OF FIGURE 4

A, liver after one subcutaneous injection of colloidal benzpyrene (mouse 207, between five and six months after injection). There is a diffuse interlobular and intralobular inflammatory reaction with considerable destruction of the liver parenchyma. The granulation tissue consists mainly of mononuclear elements derived from reticuloendothelial cells, intermingled with polymorphonuclear leukocytes.

B, a high power view of the same liver, revealing the degree of mesenchymal proliferation and destruction of the liver parenchyma itself (mouse 207).

C, liver eighteen to nineteen months after one subcutaneous injection of colloidal benzpyrene (mouse 1083). A peculiar kind of fat cell formation is present inside the broadened portal fields, showing perivascular and pericholangitic cell infiltrations. The portal fields are irregularly enlarged, with increased proliferation of connective tissue. The result is an irregular pattern of the entire field due partly to the destruction of the liver lobes and partly to connective tissue proliferation.

few cases there were seen periarteritis nodosa–like lesions with the characteristic intravascular and extravascular inflammatory and necrotic changes.

It should be mentioned that all of these changes occurring in normal mice may be due to parasites or infectious agents. In view of the

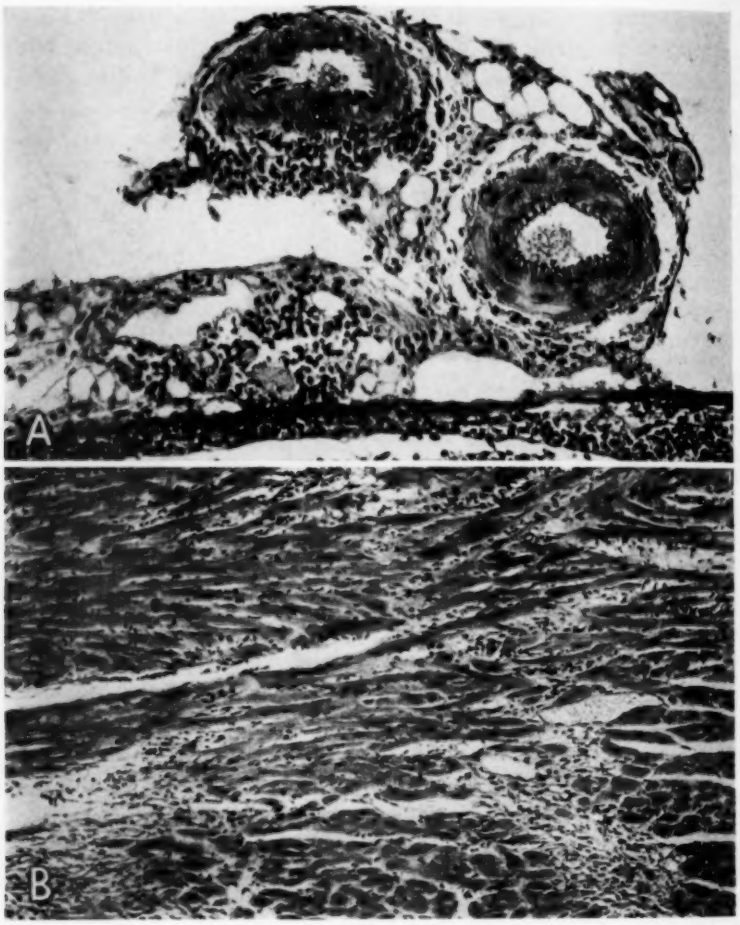

Fig. 5.—Controls. *A*, vessels of a 1 year old "normal" female Rockland mouse (no. 979) in the fat tissue surrounding lymph nodes. Two cross sections of vessels are seen with moderate lesions. One shows a thickened wall; the other shows periarteritic cell accumulations. *B*, heart of a 1 year old "normal" female Rockland mouse (no. 982) showing inflammatory foci replacing muscle fibers in the center of the ventricle (called a moderate lesion). These areas consist of some granulation tissue and some fibrosis. The tendency to scar formation is evident. No pathologic changes were found in other organs.

extensive changes in the kidneys of control mice, we considered it best not to draw any conclusions from our observations on the kidneys of the benzpyrene-treated mice.

For the same reasons we excluded our observations on the pulmonary lesions in benzpyrene-treated mice. Of the untreated mice, more than one third showed perivascular infiltrations or inflammatory foci. The percentage of lesions in the benzpyrene-treated mice was higher, and additional alterations of the alveolar septums, such as swelling and thickening, could be noted, leading to increased density and diminution of the air spaces. Even if the percentage of such lesions in the benzpyrene-treated mice was higher, the difference was not sufficiently striking to enable us to draw any conclusions with regard to the relationship between the changes due to benzpyrene and those observed in the lungs of "normal" mice.

The same is true for the tumors encountered in the lungs. In 22 per cent of the normal control mice, about 12 months of age, and in 10 per cent of the benzpyrene-treated mice, over 8 months of age, tumor-like nodules of adenomatous structure were seen in the lungs, evidently originating in the parenchyma itself. These occurred independently of tumor formation in other sites, and those occurring in tumor-bearing mice did not resemble metastases of the original tumors (sarcoma).

We therefore have the impression that there is no relationship between adenoma in the lungs and injection of benzpyrene, since the tumor formations occurred twice as often in the normal controls as in the mice given injections of benzpyrene. However, we worked only with commercial Rockland mice and are therefore unable to discuss the different observations in different strains of mice described by Campbell,[18] Bittner,[19] Andervont [20] and Lorenz and Andervont.[21]

Additional confirmation was obtained from our studies of lungs from various strains of mice with spontaneous mammary tumors. In these mice we often encountered pulmonary tumors (about 80 per cent); in about one third of the cases the pulmonary tumors did not show the histologic appearance of the original tumor of the breast but presented a benign adenoma-like structure originating in the lung. The relationship between these structures and the original mammary tumors seems therefore doubtful.

OBSERVATIONS IN BENZPYRENE-TREATED MICE

The microscopic alterations found in the benzpyrene-treated mice are shown in table 3.

18. Campbell, J. A.: Brit. J. Exper. Path. **20**:122, 1939.
19. Bittner, J. J.: Pub. Health Rep. **53**:2197, 1938.
20. Andervont, H. B.: Pub. Health Rep. **53**:1647, 1938.
21. Lorenz, E., and Andervont, H. B.: Arch. Path. **29**:484, 1940.

Inasmuch as slight lesions were encountered in untreated controls of the same sex, stock and age group, the slight changes here may be disregarded. However, in a comparison of table 2 with table 3 the high figures in the columns of the moderate and marked changes for all organs are striking in table 3. For instance, we never found marked changes in the heart, liver, gallbladder, stomach, intestines and urinary bladder of normal mice, but striking diffuse lesions were seen in the benzpyrene-treated animals (table 3).

TABLE 3.—*Incidence and Distribution of Histologic Lesions in Benzpyrene-Treated Rockland Mice (Single Subcutaneous Injection)*

Organ	Number of Organs Examined	Slight	Moderate	Marked
Lungs	267	149	56	16
Heart	270	128	48	58
Liver	276	98	121	53
Gallbladder	199	54	32	28
Fat and connective tissue of mesentery and of region around spleen and pancreas	202	76	45	26
Stomach and intestines	97	30	20	21
Kidney	243	109	95	30
Urinary bladder	119	32	40	23

TABLE 4.—*Percental Distribution of Histologic Lesions in Different Organs*

Organ	In Untreated Normal Rockland Mice			In Benzpyrene-Treated Rockland Mice		
	Slight	Moderate	Marked	Slight	Moderate	Marked
Lungs	33	10	0	55.8	21	6
Heart	31	3	0	47.4	17.8	21.4
Liver	51	15	0	33.7	43.7	19.2
Gallbladder	9	1	0	27.1	16.1	14.0
Fat and connective tissue of mesentery and of region around spleen and pancreas	16	3	1	37.6	22.2	12.8
Stomach and intestines	31	7	0	30.9	20.6	21.6
Kidney	39	36	3	44.8	39.1	12.3
Urinary bladder	37.7	7.6	0	26.9	33.6	19.3

Table 4 shows the percental distribution of lesions in different organs and demonstrates the predominance of such lesions in organs of benzpyrene-treated mice as compared with normal mice. These lesions were found more frequently in mice in which tumors did not develop than in tumor-bearing mice, as seen by the findings shown in table 5, which compares the incidence of moderate and marked visceral lesions in tumor-bearing mice and mice without tumors. The definitely increased number of lesions in the mice in which tumors did not develop is readily seen (25.2 to 63.8 per cent).

Even more pronounced is the predominance of visceral lesions in the nontumor-bearing mice compared with the tumor-bearing ones if

the animals are separated into two groups depending on whether the lard or the colloidal solution was injected (table 6).

So far an explanation cannot be offered for the fact that colloidal benzpyrene causes these visceral lesions more frequently than benzpyrene in lard, despite the fact that the amount of benzpyrene in the former was one tenth only of that of the latter. It seems probable that colloidal benzpyrene more readily reaches the lymph and blood circulation and may therefore promote or stimulate the general mesenchymal reactions. On the other hand, it is generally believed that benzpyrene dissolved in lard is fixed at the site of injection and can be found there for many months.

TABLE 5.—*Incidence of Visceral Lesions in 340 Mice Given a Single Subcutaneous Injection of Benzpyrene*

In 99 Tumor-Bearing Mice		In 241 Mice in Which Tumors Did Not Develop	
Number	Percentage	Number	Percentage
25	25.2	154	63.8

TABLE 6.—*Percental Distribution of Visceral Lesions Following Single Injection of Different Preparations of Benzpyrene*

Benzpyrene in Lard		Colloidal Benzpyrene	
Tumor-Bearing Mice	Mice in Which Tumors Did Not Develop	Tumor-Bearing Mice	Mice in Which Tumors Did Not Develop
25.0	53.0	27.2	73.3

In analyzing our results we considered the fact that lesions of a somewhat similar nature have been reported after the application of tar (Lipschütz;[22] Mertens;[23] Franco and Affonso;[24] Doederlein;[25] Fischer-Wasels;[26] Twort and Twort[7]). Franco and Affonso[24] stated that they found chronic interstitial myocarditis in some of their animals after the application of tar. They emphasized the presence of tar granules in all organs, in the blood stream, in the leukocytes, in the vascular endothelium and in Kupffer cells—however, never in the lymph nodes. Severe inflammatory reactive changes in the blood vessels, as

22. Lipschütz, B.: Ztschr. f. Krebsforsch. **21**:50, 1923.
23. Mertens, V. E.: Ztschr. f. Krebsforsch. **20**:217, 1923.
24. Franco, E. E., and Affonso, C.: Pathologica **18**:8, 1926.
25. Doederlein, G.: Ztschr. f. Krebsforsch. **23**:241, 1926.
26. Fischer-Wasels, B.: Allgemeine Geschwulstlehre, in Bethe, A.; von Bergmann, G.; Embden, G., and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie, Berlin, Julius Springer, 1927, vol. 14, sect. 2, pt. 2, p. 1726; Die allgemeine Krebsdisposition, Second International Congress of Scientific and Social Campaign Against Cancer, Bruxelles, 1936, p. 138.

periarteritis nodosa, have not been reported. For this reason the findings mentioned are not nearly as striking as ours.

Five additional cases,[26a] which were unusual, are worth reporting. In these cases splenohepatomegaly was extremely pronounced (about three times normal). The liver showed a hypertrophic stage of cirrhosis,

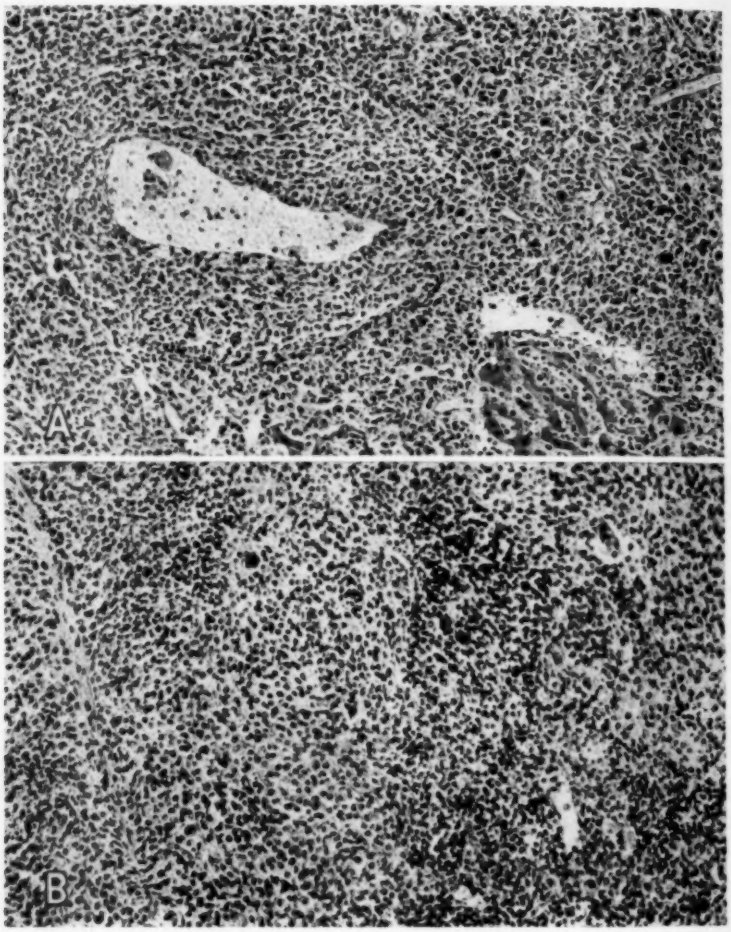

Fig. 6.—*A*, liver ten months after one subcutaneous injection of benzpyrene in lard (mouse 873). The cellular infiltration is most extensive, and there is evidence of an invasive tendency on the part of the proliferated reticuloendothelial cells. This is indicated by the infiltration into the wall of the large vessels and by the marked destruction of the hepatic parenchyma. The liver was three times larger than normal. *B*, spleen from the same mouse. Practically no normal splenic structure is to be seen. The same type of reticuloendothelial proliferation is present and displaces the normal tissues.

26a. Up to Dec. 15, 1940, 4 more such cases were observed.

with rough, uneven surface and scattered smaller or larger yellowish nodes projecting above the surface. The gallbladder showed either slight enlargement with a clear content or slight fibrosis. In all 5 cases the spleen was dumbbell-shaped, with some infarct-like necrotic areas at the margin; the weight ranged from 0.7 to 1.1 Gm. Ascites always was present.

Microscopically, in these cases, a complete change of structure of the hepatic parenchyma had occurred. Most of the parenchyma was replaced by extensively proliferated mesenchymal tissue, derived from the reticuloendothelial system and consisting mostly of large mononuclear elements and reticular fibers. Only a few remaining liver cell cords or liver cells could be seen. The invasive character of this proliferating tissue was indicated by the infiltration into the walls of large vessels and by the marked destruction of the hepatic parenchyma itself (fig. 6*A*). A similar change took place in the spleen. The normal structure was replaced by a diffuse reticular proliferation and the lymph follicles could not be recognized (fig. 6*B*).

The etiologic factors involved and their mode of action have not yet been ascertained. The question arises whether these widespread alterations result from the absorption of benzpyrene and its elimination from the body directly or indirectly by converting the tissues or whether a latent infectious agent frequently present in normal mice was stimulated by the presence of this toxic substance or by some sensitizing mechanism. A number of routine bacteriologic examinations gave negative results. However, virus studies were not done. Recent investigations (Sabin;[27] Swift and Brown;[28] Horsfall and Hahn[29]) have established the fact that mice are frequently the carriers of a virus. We consider it possible, therefore, that such toxic substances as benzpyrene when injected into normal "healthy" mice may stimulate a virus already present and thus cause the lesions described. The full understanding of the mode of action, however, must await the results of systematic virus and biochemical studies in connection with carcinogenic substances.

SUMMARY

After a single injection of benzpyrene in mice, generalized vascular and visceral lesions were observed, such as periarteritis nodosa, diffuse myocarditis and extensive mesenchymal proliferation, which were apparently of an inflammatory nature. Particularly notable were the latter lesions in the liver, spleen and lymph nodes. These lesions occurred more frequently in the benzpyrene-treated mice in which tumors failed to develop and were not observed in control mice of the same stock and age.

27. Sabin, A. B.: Science **90**:218, 1939.
28. Swift, H., and Brown, T. M.: Science **89**:272, 1939.
29. Horsfall, F. L., and Hahn, R. G.: J. Exper. Med. **71**:391, 1940.

Case Reports

FIBROSARCOMA OF THE DIAPHRAGM

Report of a Case with Review of the Literature

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The literature furnishes reports of but 17 primary tumors of the diaphragm, both benign and malignant. The benign tumors were diagnosed as lipoma (3),[1] angiofibroma (1),[2] chondroma (1) [3] and fibroma (1).[4] The tumors reported by Bonamy [5] as fibromyoma were described by his consultant, Philibert, as pure fibroma. The malignant primary tumors of the diaphragm reported were diagnosed as myogenic sarcoma (5),[6] round cell sarcoma (1) [7] and "fibromyosarcoma" (1).[8] Another tumor diagnosed as sarcoma, its nature not specified, was mentioned by Borchard [9] in a discussion of diaphragmatic resections.

Some of the primary diaphragmatic tumors reported as malignant do not stand close scrutiny and may be considered benign. Among them is the only human tumor reported under the name of fibrosarcoma of the diaphragm (Alexander [10]). This tumor seems benign from both the gross description of it and the drawing of its structure. A large growth described by Van Nes [11] as a fibrosarcoma had for its only malignant feature slight irregularity in the size of the cells. Not included among the 17 primary tumors of the diaphragm, all of which were found in human beings, were 2 diaphragmatic tumors found in a cow reported by Granucci [12] as fibrosarcoma; the descriptions are not unequivocal and

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2. Burvill-Holmes, E., and Brody, W.: Am. J. M. Sc. **183**:679, 1932.
3. Kramer, S. P.: Virchows Arch. f. path. Anat. **156**:188, 1899.
4. Granché, M.: Bull. Soc. anat. de Paris **43**:385, 1868.
5. Bonamy, R.: Paris chir. **4**:1051, 1912.
6. Müller, W.: Centralbl. f. allg. Path. u. path. Anat. **58**:353, 1933. Kirshbaum, J. D.: Am. J. Cancer **25**:730, 1935. Peery, T. M., and Smith, W. A.: ibid. **35**:416, 1939. Ryan, E. J.: Cleveland Clin. Quart. **6**:304, 1939.
7. Dalzell: Glasgow M. J. **27**:298, 1887.
8. Sauerbruch, F., and O'Shaughnessy, L.: Thoracic Surgery, Baltimore, William Wood & Company, 1937, p. 353.
9. Borchard, A.: Verhhandl. d. deutsch. Gesellsch f. Chir. **41** (pt. 1): 160, 1912.
10. Alexander, B.: Magyar orvosi arch. **5**:54, 1896.
11. Van Nes, C. P.: Nederl. tijdschr. v. geneesk. **65**:583, 1921.
12. Granucci, L.: Gior. d. r. Soc. ed Accad. vet. ital. **52**:892, 1903.

the growths may well have been benign. A diagnosis of primary diaphragmatic tumor based on roentgenographic study alone cannot be accepted.[13]

The following report of a primary tumor of the diaphragm is justified by the fact that nothing like the tumor has been found described in the available literature.

REPORT OF CASE

A 73 year old white woman admitted to the service of Dr. M. Rabinowitz at the Jewish Hospital of Brooklyn, April 4, 1937, stated that three weeks previously she had noticed swelling of the feet. When the edema had mounted to the knees she was put to bed and then the edema gradually subsided. After a few days, she felt weak, lost her appetite and vomited occasionally. During the last two weeks before admission she was costive and was relieved only by enemas. For a week before admission she had hiccuped frequently, and in the last four days she had become unusually thirsty. There had been no recent loss of weight.

The temperature was 99.6 F., the pulse rate 100 and the respirations 36. The blood pressure was 130 mm. of mercury systolic and 94 mm. diastolic. The skin was cold and moist and the cheeks slightly flushed. The tongue was heavily coated; the breath, malodorous. The right lobe of the thyroid gland was enlarged, and the trachea deviated to the left. There was flatness to percussion over the base of the right lung posteriorly, and no transmission of voice or breath sounds. The percussion note over the base of the left lung was dull, and the transmission of breath sounds was depressed. The maximum cardiac impulse was obtained in the fifth left intercostal space near the anterior axillary line. A systolic murmur was heard at the apex. The abdomen was markedly distended. The abdominal wall, the back and the lower extremities were edematous. Both costovertebral angles were tender.

The roentgenographic markings of both lungs were exaggerated at the bases, and it was suspected that there was a small amount of fluid on the right side. Roentgenograms revealed hypermotility of the stomach and intestines and poor filling of the duodenal cap. April 21 the patient became stuporous. The next day her right hand and forearm became swollen and cyanotic. She remained comatose until her death, later that night. She had been in the hospital for eighteen days.

The urine was acid and contained some albumin and occasional red blood cells, white blood cells and granular casts; its specific gravity was 1.020. The hemoglobin content was 88 per cent (Sahli), and the red blood cell count was 4,600,000. There were 22,000 leukocytes per cubic millimeter, 83 per cent of which were polymorphonuclear neutrophils. The blood sedimentation rate varied from 13 to 3 mm. per hour. The blood sugar was 137 mg. per hundred cubic centimeters, the total protein 5.57 Gm., with an albumin globulin ratio of 0.92, the urea nitrogen 50 mg., the creatinine 1.4 mg. and the chlorides 311 mg. The icterus index was 17.4. The Kline flocculation test for syphilis was negative. The benzidine test for blood in the stool was negative. The circulation time by the saccharine method was sixteen seconds. April 17, the venous pressure was 12 cm. of water in the left arm, 14.5 cm. in the right arm and 38 cm. in the left leg.

The clinical diagnoses were cardiac decompensation and obstruction of the inferior vena cava.

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13. Gravano, L.: Semana méd. **2**:705, 1935. Spangenberg, J. J.; Gattini, H., and Sloer, M.: Prensa méd. argent. **22**:17, 1935.

Necropsy (ten hours after death).—The body was that of a well developed woman 143 cm. tall and weighing about 55 Kg. There was pitting edema of both lower extremities, of the trunk to the axillas and of the right hand. The skin and scleras had a faint yellow tint. There were a few prominent veins in the skin of both lower quadrants of the abdomen, but no caput medusae. The adipose tissue in the midline of the anterior abdominal wall was 1.5 cm. thick. The diaphragm was at the level of the fifth rib on the right side and at that of the fifth intercostal space on the left.

Behind the liver, in the region of the right crus of the diaphragm was a tumor of irregular shape, measuring 8 cm. from side to side, 7.5 cm. anteroposteriorly and 5 cm. from above down. The mass was of almost ligneous hardness and was fixed to the surrounding structures. Above, it was fused with the lower lobe of the right lung over an area 7 by 5 cm., and anteriorly, with the posterior aspect of the liver, surrounding the inferior vena cava. Into the middle of its right side passed the medial portion of the right dome of the diaphragm, and over its left side disappeared the left dome of the diaphragm. The right adrenal gland was embedded in, and concealed by, the anterior and inferior portion of the tumor. The left lateral aspect of the mass was marked by nodular protuberances up to 2 cm. in diameter. The vessels to the right kidney coursed along the anterior aspect of the mass, and the celiac and superior mesenteric arteries were displaced a little to the left and lay in grooves on the tumor's left and inferior surfaces. The attachments of the tumor to the central portion of the diaphragm, to the liver and the adjacent inferior vena cava and to the right lung and right adrenal gland were inseparable. The attachments to the celiac, superior mesenteric and right renal vessels were easily sundered. The aorta was not affected.

The hepatic segment of the inferior vena cava was displaced forward by the tumor, and its diaphragmatic segment was buried within the mass. The orifice of the inferior vena cava into the right atrium of the heart was not unusual. Below this, the vessel was filled with pale red firm blood clot, which extended back into the hepatic veins. In the left renal vein and in the lower portion of the right ovarian vein there was dark red clotted blood.

The cut surfaces of the tumor were white; in some areas were faint striae in whorls, and elsewhere, especially where the diaphragm disappeared into the tumor, were coarser striae in a parallel rectilinear pattern. In places, circumscribed smooth white areas and small yellow areas were noted. The cut surfaces of the nodular protuberances showed a few small pale red areas. The architecture of the diaphragmatic muscle near its disappearance into the mass was obliterated by white streaks.

The right pleural cavity contained about 200 cc. of clear yellow fluid; the left was obliterated by fibrous adhesions. The heart weighed 280 Gm. and presented nothing remarkable. The lungs together weighed over 1,300 Gm. and presented patches of pneumonia. The spleen was firm and weighed 150 Gm. The liver weighed about 1,650 Gm., and in its cut surfaces there were a number of hepatic veins filled with dark red, friable clotted blood. The large bile ducts were patent.

The right adrenal gland was embedded in the tumor. It was not unusual in size or shape or in the appearance of its cut surfaces. The left adrenal gland weighed 5 Gm. The kidneys were not remarkable. The endometrial surface of the uterus was dark red, and beneath it the tissue was infiltrated with blood. Some of the vessels in the broad ligament were filled with clotted blood. The thyroid gland weighed 42 Gm. and contained a soft adenoma in its right lobe. The tracheobronchial lymph nodes were soft, black and measured up to 2 by 1.3 cm. Near the tumor, below the diaphragm there were a few gray and black lymph nodes measuring up to 1 by 0.5 cm.

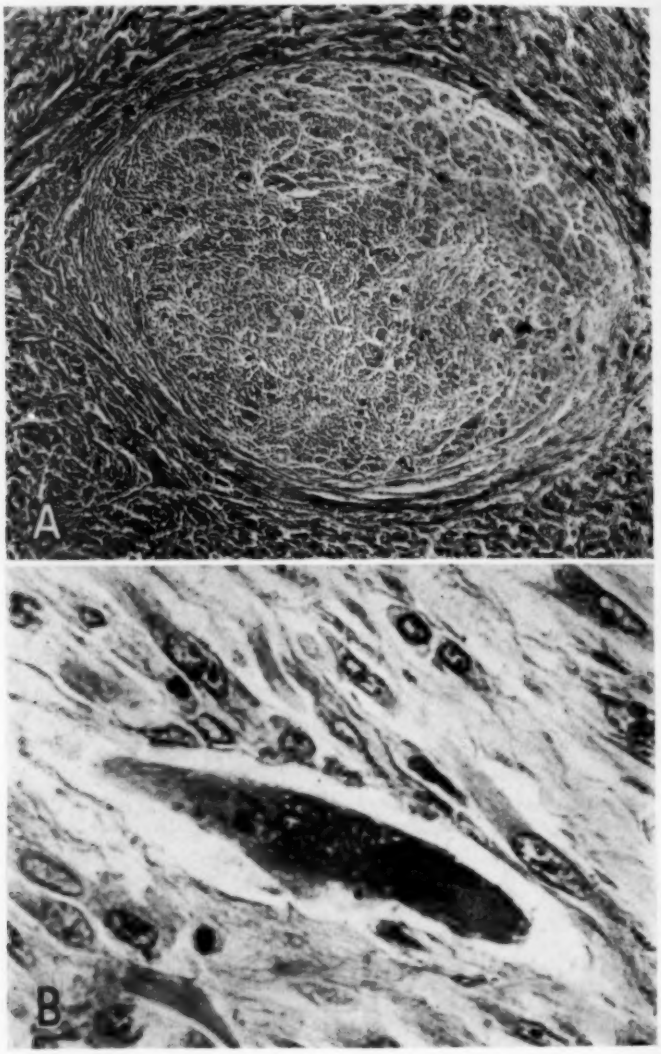
Various parts of the tumor were fixed in solution of formaldehyde U. S. P. or in Zenker-acetic acid solution and stained with hematoxylin-eosin, Mallory's phosphotungstic acid–hematoxylin, a combined Van Gieson-Weigert elastic tissue stain and Laidlaw's connective tissue stain.

Microscopic Examination.—A portion of the tumor adjacent to the liver consisted of interlacing bundles of fibers and elongated nuclei, in places in a whorl-like arrangement (fig. *A*). In one area the fibers were long and slender, resembling those of smooth muscle, and the nuclei, centrally placed, were plump or slender, vesicular or deep staining. Near by, the tissue was loose and fibrillar, and the nuclei were thinner and darker. Here, too, were extensive areas of extravasated blood about distended blood vessels. Many of the capillaries were filled with clotted blood. In other areas the fibers were coarse, resembling collagenous connective tissue fibers, and the nuclei were often irregular in size, shape and depth of stain. Still other portions consisted of dense masses of hyalinized fibers, with few nuclei. Here and there sparse collections of spindle-shaped cells with large oval vesicular nuclei were noted. With Van Gieson's method, the fibers stained red, and the scattered plump or slender spindle-shaped cells stained pale yellow. In some places, spindle-shaped cells with deep-staining nuclei, some large, oval and vesicular and some slender, were arranged in close layers separated by a pink or faintly blue-staining granular or finely fibrillar material. In looser areas these layers were separated by clefts containing red blood cells and polymorphonuclear leukocytes. Between the tumor and the adjacent liver tissue there was a zone of fibrous connective tissue containing large, thin-walled blood vessels and accumulations of large mononuclear cells.

In another region, among the spindle-shaped cells or surrounded by more dense, vascular fibrous tissue were large cells with barely discernible or imperceptible outlines. The more discrete cells were round or markedly irregular in shape. Some were crudely stellate and others round or oval with a short tapering extremity. A few were pointed at each end, and an occasional one was pear or club shaped. The cytoplasm was pink, and frequently blended with that of the surrounding fibers. In a few cells it was faintly blue staining and finely granular. The largest cells measured (with the micrometer eyepiece) up to 102 by 23 microns (fig. *B*). The nuclei varied up to 20 by 8 microns. The smaller ones were often deep staining and irregular, with no distinct structure; some were multiple. Those of larger size were round, oval or elliptic. In many, the chromatin was sparse and scattered in irregular granules. In a few there were nucleoli reaching 4 or 5 microns in diameter. An occasional cell contained two round vesicular nuclei with large nucleoli. The position of the nucleus in the cell was central, paracentral or peripheral. When nuclei were multiple, they had no regular arrangement, sometimes occupying the central part of a round cell and sometimes the center or the end of an elongated cell. In one round tumor cell, 12.5 microns in diameter, the nucleus was in a state of mitotic division.

In another preparation a broad zone of dense hyalinizing connective tissue continuous with the tumor separated the diaphragmatic muscle bundles from the liver tissue. The muscle fibers and the liver cells stained yellow-green with the combined Van Gieson-Weigert elastica method, and the tumor tissue stained red except for scattered pale yellow cells. In a portion of the tumor including the diaphragm the muscle fibers were shrunken and gradually disappeared within the mass. The transverse striae of the muscle fibers were not visible, and their nuclei were small and deep staining. Near by, there were large accumulations of small round cells and large mononuclear cells. The fibers of this section of

tumor were coarse and hyalinizing, and had few wizened nuclei. In places there were deposits of lavender-staining granules. With the Van Gieson-Weigert elastica stain, faint transverse striations were visible in a few of the diaphragmatic muscle fibers close to the tumor. Deeper in the tumor tissue there were a few



A, whorl-like arrangement of the fiber bundles in a portion of the tumor near the liver. Mallory's phosphotungstic acid and hematoxylin stain; × 104. *B*, giant cell among other varieties of cells and fibers. Hematoxylin and eosin stain; × 647.

scattered fragments of protoplasm having no cellular detail and staining like the indistinct remnants of muscle fibers nearer the periphery.

In another portion of the tumor, close to the diaphragm, there were embedded a number of large nerve fibers and a lymph node. The connective tissue of the lymph node merged with that of the tumor. The lymph sinuses were distended with large mononuclear cells, and the connective tissue septums were impregnated with coarse black particles and fine granules.

In a section of the mass adjacent to the right lung there was a tongue of pulmonary tissue, caught and compressed by the tumor tissue. The lung was separated from the mass, in a combined Van Gieson-Weigert elastica preparation, by a broad band of red-staining fibrous connective tissue with several interrupted layers of black-staining elastic fibers. Fragments of elastic fibers were scattered through the tumor tissue. With this stain, a few large polyhedral tumor cells had yellow cytoplasm and vesicular nuclei. Prepared with phosphotungstic acid, the fibers of the mass stained deep red and the nuclear material blue, except for scattered large polyhedral cells with pale blue–staining cytoplasm and one or a few large irregular nuclei.

The capsule of the right adrenal gland was broad and hyalinizing and was surrounded by the dense tissue of the tumor.

Portions of the inferior vena cava presented slight infiltration of the media and adventitia with small round cells. In places the smooth muscle bundles were missing and the dense fibrous tissue of the vessel wall merged with that of the adjacent tumor tissue. In the latter there were embedded numerous adipose tissue cells, some nerve fibers and ganglions.

The liver sinusoids were distended with blood, and most of the liver cell cords in the central half of each lobule were obliterated.

The connective tissue between the lobules of the pancreas was increased and infiltrated with small round cells, large mononuclear cells and some polymorphonuclear leukocytes. A similar cellular infiltration was noted in the peripancreatic adipose tissue. In the kidneys a few glomeruli were hyalinized, and scattered areas of increased connective tissue in the cortex were infiltrated with small round cells.

Anatomic Diagnosis.—Fibrosarcoma of the diaphragmatic tendon on the right; thrombosis of the inferior vena cava and tributaries; edema of the trunk and lower extremities; passive congestion, hemorrhage and necrosis of the liver; hemorrhage in the endometrium; tracheitis, bronchitis and bilateral focal pneumonia; interstitial pancreatitis; adipose tissue infiltration of the myocardium; hydrothorax on the right; healed tuberculous nodule in a tracheobronchial lymph node; fibrous pleural and peritoneal adhesions; adenoma of the thyroid gland; ossification of the cricoid cartilage; sclerosis of the abdominal portion of the aorta; cysts of the kidneys; anomaly of the pelvis of the left kidney; double ovarian vein on the left; polyp of the uterine cervix; cysts of the broad ligament.

COMMENT

The death of the patient was caused by thrombosis of the inferior vena cava, which followed compression of the vein by the tumor. First, the caval obstruction produced edema of the lower extremities. Then it interfered with the venous drainage of the liver, causing anorexia, vomiting and slight jaundice. Finally, it hampered blood flow from the kidneys, permitting nitrogenous retention and easing the patient into coma. Clinically, therefore, the tumor was malignant by position.

Pathologically, the tumor was considered malignant because of its gross as well as its microscopic appearance. It had invaded, albeit

feebly, the diaphragm and the right lung. Also, it had no discrete capsule and had become fused, over broad areas, with the liver, vena cava and right adrenal gland. There were no distant metastases, yet malignancy is not thereby denied, for the absence of metastatic deposits is common with even larger and more vigorous cancerous growths. Moreover, even histologically benign tumors have recently been accused of forming metastases.[14] The irregularity of cell size, shape and arrangement, a prominent microscopic feature of this tumor, firmly supports the claim of malignancy.

Now, where did the tumor arise? Its position and the way it involved adjacent organs both above and below the diaphragm point to that structure as the source. The histologic appearance of the tumor identifies it as a derivative of connective tissue. It must have arisen, therefore, from muscular, nerve or tendinous elements. Nowhere did the tumor cells present the fibrillar or striate distribution of cytoplasmic granules which might have suggested smooth or striped muscle as the mother tissue, nor were there any centrioles such as Wolbach depicted in a case of malignant rhabdomyoma.[15] An origin of the tumor from connective tissue associated with the nervous system was excluded by a study directed specifically to that question (I. M. Tarlov). This leaves the connective tissue of the diaphragm, probably the tendinous center, as the most likely source of the tumor.

SUMMARY

A fibrosarcoma of the diaphragm, of which no counterpart can be found recorded in the available literature, is reported, together with references to all the descriptions of primary diaphragmatic tumors so far published.

14. Jaffé, R. H.: Arch. Path. **25**:60, 1938.
15. Wolbach, S. B.: Arch. Path. **5**:775, 1928.

THROMBOSIS OF THE PULMONARY ARTERY IN IDENTICAL TWINS

Report of Cases with Autopsy

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In 1897 Dickinson [1] reported a fatal case of thrombosis of the trunk and main branches of the pulmonary artery in a boy 4½ years of age, but no similar cases of this condition in children have been recorded in the available literature. Thrombosis of the trunk or main branches of the pulmonary artery has been seen in a number of adults, as shown by the reviews of Fowler [2] and Kampmeier.[3] Since the publication of those reviews, cases have been reported by Desclin and Regnier,[4] Montgomery,[5] Pou and Charr,[6] Barsoum,[7] Burke [8] and others. As an incidental finding, thrombosis of the smaller radicals of the pulmonary artery is observed commonly enough at the autopsy table.

The cases which are reported here are of interest not only because thrombosis of the pulmonary artery is rare in children but because the patients were identical twins, in whom the accidents occurred almost simultaneously.

REPORT OF CASES

The twins were identical white boys, 15 months of age. The weight of each at birth was approximately 3¼ pounds (1,420 Gm.), birth occurring in the seventh month of pregnancy. The weight of each at the time of death was about 15 pounds (6.8 Kg.). They died nine days apart.

Twin 1.—For four or five months preceding death, considerable feeding difficulty had been encountered. Vomiting, diarrhea and fever began five days before death, and the child cried as if in pain. The vomiting persisted, and dehydration with acidosis resulted. The ears, throat and chest were normal, but some abdominal rigidity was present. A roentgenogram of the chest was reported as essentially normal. The clinical impressions were dehydration, acidosis and colitis. Death occurred two days after hospitalization, during administration of fluids. For about a day before death some serious respiratory abnormality had been present, but its nature was not clear.

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1. Dickinson, L.: Tr. Path. Soc. London **48**:57, 1896-1897.
2. Fowler, W. M.: Ann. Int. Med. **7**:1101, 1934.
3. Kampmeier, R. H.: J. Thoracic Surg. **3**:513, 1934.
4. Desclin, L., and Regnier, M.: Arch. d. mal. du cœur **24**:726, 1931.
5. Montgomery, G. L.: J. Path. & Bact. **41**:221, 1935.
6. Pou, J. F., and Charr, R.: Am. Rev. Tuberc. **37**:394, 1938.
7. Barsoum, H.: Brit. M. J. **2**:620, 1938.
8. Burke, M.: Am. J. M. Sc. **196**:796, 1938.

Autopsy.—The external examination revealed nothing unusual, and the body cavities were normal. As the pulmonary artery was opened, a mass of thrombotic material fell out of it. This mass measured 1 cm. in length and about 1 cm. in diameter—a size which indicated that it had come from a vessel about equal in diameter to a major branch of the pulmonary artery. Further investigation revealed that the right pulmonary artery was completely occluded by a fresh thrombus and that numerous branches of the left pulmonary artery were similarly affected. On careful study of the plugs it seemed that they had arisen in the pulmonary system, but no explanation of their occurrence could be offered, for the vessel walls showed no gross abnormalities. There was definite dilatation of the right side of the heart; otherwise this organ seemed normal. Nothing of significance was found in the gastrointestinal tract or in the remainder of the body. No pus was encountered in removing the petrous and mastoid portions of the temporal bones.

Microscopic Examination.—In a full section of the right branch of the pulmonary artery was a thrombus which almost completely occluded the lumen of the vessel. In some places this thrombus was attached to the wall of the artery; in other places it was free. In the small free spaces fresh blood was seen. The structure of the thrombus was typical, with branching and anastomosing sheets of disintegrating platelets, connected by festoons of fibrin, in which leukocytes and erythrocytes were entrapped. Many of the erythrocytes had partially or completely lost their color. Along the line of attachment to the vessel wall organization had begun to take place. The pulmonary artery, however, showed no evidence of inflammation or any other lesion which might have resulted in thrombosis. The appearance of the thrombus did not suggest that it was infected, and a bacterial stain revealed no micro-organisms. Sections of the right lung showed that the thrombus continued for a considerable distance into the branches of the pulmonary artery. Here also organization was observed in several places. The smaller vessels were plugged with propagated clot. In sections of the left lung some of the vessels were occupied by thrombi also. Infarction had not occurred, but a few of the alveoli contained fluid. Contrary to the gross appearance, sections of the ears showed acute purulent otitis media. The other organs and tissues showed no lesions.

Anatomic Diagnosis.—Acute otitis media; thrombosis of the right pulmonary artery and of the secondary branches of the left pulmonary artery; dilatation of the right side of the heart; lobular pneumonia.

Twin 2.—About three weeks before death this child presented vomiting, diarrhea and fever. Four days later a purulent discharge from the left ear appeared, and the drum was incised. The condition did not improve, and the patient was hospitalized. The urine contained many casts and occasional red and white blood cells. The white blood cells numbered 15,000, with 72 per cent neutrophils. In spite of fluids and transfusions, the child gradually became weaker. Mastoidectomy on the left side was followed by marked respiratory embarrassment, and death occurred in about five and one-half hours.

Autopsy.—The body had been embalmed. Externally there was nothing remarkable except the incisions for embalming and the incision for mastoidectomy. The right lung had received none of the embalming fluid, but the left lung was well injected. No thrombi were found in the superficial or deep jugular veins. The left internal jugular vein was explored as far as the base of the skull because of the mastoiditis. No thrombi were found in the right side of the heart, the venae cavae or the main trunk of the pulmonary artery. In the right major branch of the pulmonary artery was found a relatively fresh thrombus, which completely occluded its lumen and projected into the main trunk. Dissection of the right lung

showed that practically every branch of the pulmonary artery was occluded by fresh thrombus. The pulmonary artery supplying the left lung contained no thrombi. The remainder of the body revealed no significant abnormalities.

Microscopic Examination.—A section of the right main branch of the pulmonary artery showed it to be completely occluded by a thrombus of fibrin, in which were cords of leukocytes and columns of disintegrating platelets. All of the erythrocytes had disintegrated. The wall seemed normal except for the connective tissue which had begun to grow from its intima into the thrombus. There was no indication that the thrombus had arisen as a result of an inflammatory lesion in the wall. No evidence of infection and no micro-organisms were seen in the thrombus. Sections of the right lung showed thrombi of the same character in its vessels. The lungs showed lobular pneumonia with numerous gram-positive cocci in chains. A section made from curettings of the left mastoid wound contained fragments of bone and chronic granulation tissue together with some purulent exudate, in which gram-positive cocci were seen. The other sections showed nothing of significance.

Anatomic Diagnosis.—Chronic mastoiditis on the left side with purulent exudate; thrombosis of the right pulmonary artery; lobular pneumonia.

COMMENT

In certain features of their illnesses these twins were strikingly similar. Each was undernourished, and each had presented feeding difficulties. With onsets which were almost simultaneous, the final illnesses of these patients were ushered in with identical symptoms—vomiting, diarrhea and fever. In each twin respiratory embarrassment developed before death, and their deaths occurred only nine days apart. At autopsy one showed acute otitis media, and the other showed purulent mastoiditis. In each the right main branch of the pulmonary artery was occluded by a thrombus.

These unusual similarities lead one to speculate on the probable cause and genesis of pulmonary thrombosis in these twins and on the part played by the fact that they were identical twins. It is well known that similar simultaneous symmetric pathologic processes often occur in identical twins. In an excellent review of the literature on this subject McFarland and Meade [9] collected a large number of such instances, illustrating a variety of pathologic processes, but mentioned no cases of thrombosis of the pulmonary artery or of thrombosis elsewhere.

In reporting Dupuytren's contracture in identical twins, Couch [10] stated that there is a "strange common destiny in matters of life and growth which surrounds twins. . . . The characteristics which identical twins possess in common are far more deep-rooted and important than mere similarity of appearance, colour of eyes, shape of nose, curly hair, or even temperament. Elemental biological qualities governing life, growth, and even death itself are also identical." While thrombosis per se would seem to have no relation to heredity, the course of events in each of the present cases was entirely too similar and too unusual to be merely coincidental.

The simultaneous occurrence of middle ear and mastoid infections seems plausible enough. One is not surprised that 2 infants with almost

9. McFarland, J., and Meade, T. S.: Am. J. M. Sc. **184**:66, 1932.
10. Couch, H.: Canad. M. A. J. **39**:225, 1938.

identical constitutions and exposed to the same environmental factors should contract similar infections. Their resistance to infection was doubtless lowered by poor nutrition. Perhaps also each lost his congenital immunity to the organisms responsible at about the same time.

It is more difficult to explain why such patients should have thrombosis of the pulmonary arteries. As pointed out by numerous authors, the factors which seem to be of major importance in the etiology of pulmonary thrombosis are (1) sclerosis of the pulmonary artery, (2) embolism, (3) septicemia, (4) chronic diseases of the lungs, such as tuberculosis and bronchiectasis, and (5) slowing of the blood stream as from strict rest in bed and cardiac failure. In the present cases there was no gross or microscopic evidence of disease in the vessel walls, such as sclerosis or inflammation from chronic infection of the lungs, and cardiac failure was not present. Perhaps septicemia was present, and perhaps also the blood stream was slowed from rest in bed.

There remains the possibility of embolism. A small embolus may lodge in a branch too small to give the characteristic symptoms of pulmonary embolism. But, as a result of the embolism, thrombosis may occur and extend gradually to involve the main branches of the pulmonary artery. In 8 of the 60 cases collected by Fowler [2] thrombosis was found elsewhere in the venous system. Möller [11] expressed the belief that an embolic origin should be assumed in all cases and that this possibility should not be discarded until all possible sources of emboli have been excluded.

In the present cases, although the jugular veins were explored and found to be free from thrombosis, it is entirely possible that some of the small veins in the vicinity of the affected ears might have contained thrombi which were overlooked grossly but from which small emboli sufficient to produce thrombosis in the pulmonary arteries might have arisen.

In the other case in childhood, reported by Dickinson,[1] the same embolic mechanism probably was present. The 4½ year old boy showed congenital pulmonary stenosis, with vegetations on the pulmonary valves. The pulmonary artery was patent for a short distance beyond, and then was obstructed in its main trunk and primary branches by firm laminated clot.

SUMMARY

Thrombosis in one of the main branches of the pulmonary artery is reported in each of young identical twins. It is suggested that the thrombosis in the pulmonary arteries probably followed embolism from small thrombosed veins in the region of the infected middle ear in one twin and the region of the mastoid process in the other, even though such thrombosed veins were not demonstrated at autopsy.

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11. Möller, P.: Beitr. z. path. Anat. u. z. allg. Path. **71**:26, 1923.

Laboratory Methods and Technical Notes

METHOD FOR FUCHSIN STAINING OF THE NETWORK OF CEREBRAL BLOOD VESSELS

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There is little doubt that the Pickworth benzedrine method[1] of demonstrating blood vessels is a very useful procedure for neuropathologic studies. That method, however, can be applied only to fresh material which has been fixed in solution of formaldehyde U. S. P. for a few days. In brains fixed in solution of formaldehyde for months or years, the results are poor. Moreover, the Pickworth preparations often fade rapidly; hence they cannot be studied after a few weeks. The attempt was therefore justified to devise a method of demonstrating the network of cerebral blood vessels which could be applied to old material and which would give permanent preparations.

Acid fuchsin is a suitable dye for staining erythrocytes. Mandelstamm,[2] in fact, devised a method for demonstrating blood vessels, using 1 per cent solution of acid fuchsin and combining this procedure with Mallory's procedure. With that method, however, the network of vessels cannot be well visualized, because of the thinness of the sections. It is obvious that in order to demonstrate the network of vessels as in the Pickworth method, thick sections are necessary. However, these thick sections take up the dye to such an extent that it is difficult to differentiate the stain and to obtain, consequently, a clear contrast.

The present method overcomes this difficulty. It is suitable for staining frozen, paraffin and celloidin (a preparation of pyroxylin) sections. The material has to be well fixed in a 10 per cent solution of formaldehyde. The sections are to be cut 120 to 150 microns thick. The essence of the method is as follows: After preliminary acidification, the sections are stained in a very dilute solution of acid fuchsin and differentiated with a mixture of 1 per cent phenol solution and lithium carbonate. After differentiation, they are again acidified in hydrochloric acid. The network of vessels is thus demonstrated sharply, making a striking contrast to the areas devoid of blood vessels.

The details of the procedure are as follows:

The sections after removal from distilled water are acidified in a dilute solution of hydrochloric acid (3 drops of concentrated hydrochloric acid added to 150 cc. of distilled water) for three to four hours. After this preliminary acidification, they are stained in a very dilute aqueous solution of acid fuchsin, made by adding

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1. Pickworth, F. A.: J. Anat. **79**:62, 1934. Alexander, L., and Putnam, T. J.: A. Research Nerv. & Ment. Dis., Proc. (1937) **18**:471, 1938.

2. Mandelstamm, M.: Ztschr. f. d. ges. Neurol. u. Psychiat. **155**:248, 1936.

5 to 6 drops of 1 per cent acid fuchsin to 150 cc. of distilled water. The sections are stained in large dishes, care being taken that they do not cover each other. After twelve to fifteen hours, the sections are placed in a 1 per cent solution of phenol for one to two minutes and are then differentiated in 150 cc. of a

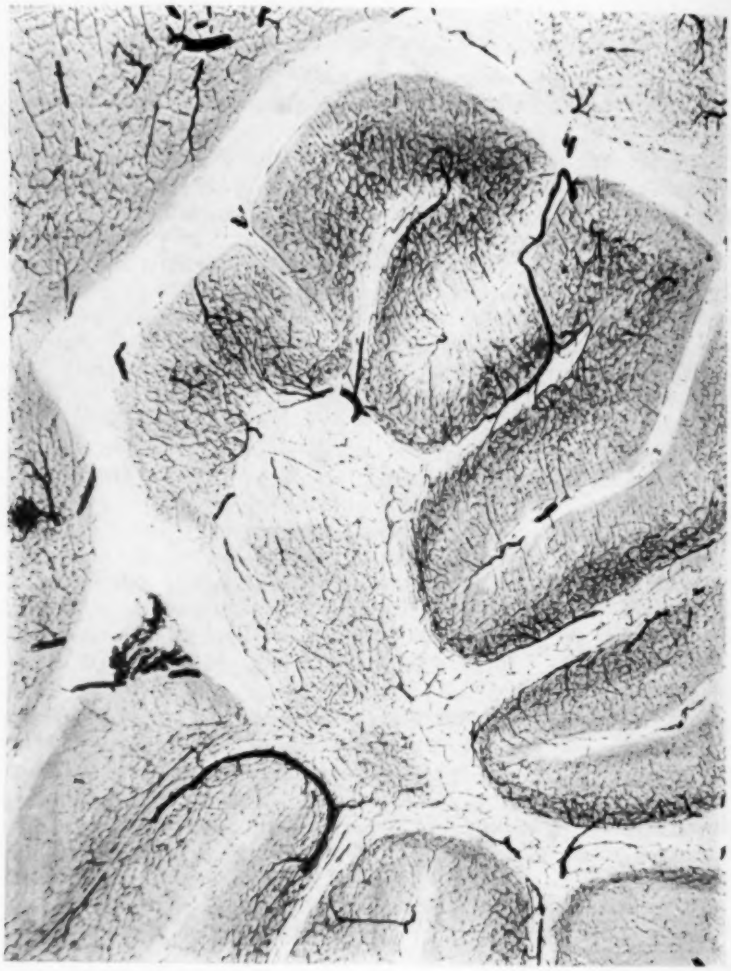

Fig. 1.—Network of blood vessels in a rat's brain as shown by the acid fuchsin method; × 40.

1 per cent solution of phenol to which 2 to 5 drops of 0.5 per cent lithium carbonate have been added. The differentiation, watched under the microscope, must be continued until the network of vessels becomes apparent (five to sixty minutes). Overdifferentiation should be carefully avoided. The sections are then washed for a short time in two changes of distilled water and from the distilled water are transferred into a diluted aqueous solution of hydrochloric

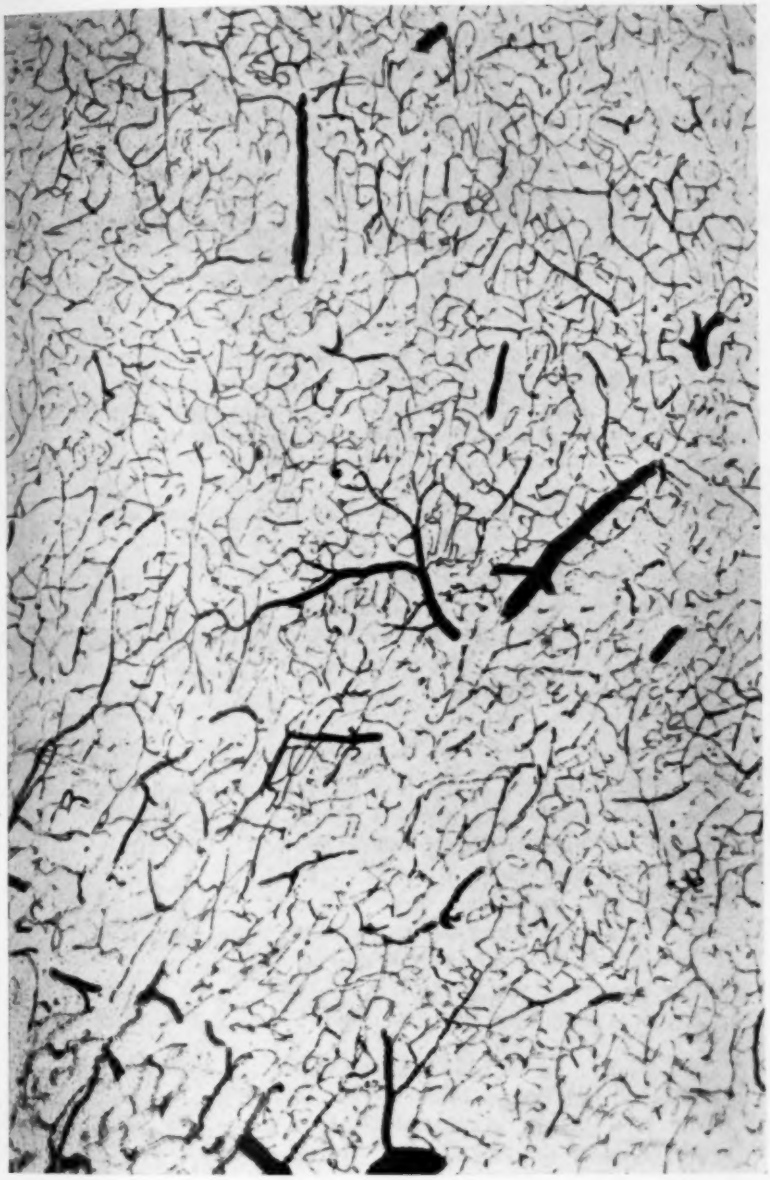

Fig. 2.—Network of blood vessels in a human brain (occipital lobe) as shown by the acid fuchsin method; × 50.

acid for ten minutes (3 drops of concentrated hydrochloric acid added to 150 cc. of distilled water). Finally, the sections are again washed in two changes of distilled water, dehydrated in alcohol, cleared in 1 per cent carbolxylene and mounted in balsam. (Sometimes when the slides are not acidified enough, they fade.)

As good results are obtained with paraffin and celloidin sections as with frozen sections. In the case of paraffin or celloidin sections the paraffin or celloidin has to be dissolved with xylene and acetone, respectively. Both paraffin and celloidin sections are mounted on the slide only after staining. With celloidin sections, it is more difficult to differentiate the ground substance of the white matter than with frozen and paraffin sections. In celloidin sections the ground substance of the white matter is reddish even after longer differentiation.

Paraffin sections are put directly into xylene and then into absolute alcohol. If the paraffin block is too fragile to be cut at 120 microns, the paraffin is dissolved with xylene, and frozen sections are cut after hydration of the block. To dissolve and hydrate paraffin sections, the following procedure is followed: The blocks are put into a 56 C. thermostat for three hours, then in first xylene for five hours, second xylene for twelve hours, first acetone for five hours and second acetone for twelve hours, and then transferred into water.

Usually, I successfully use the following combined method for embedding and cutting:

A. Embedding in paraffin of material fixed in solution of formaldehyde, as follows:

First acetone—one to one-half hour
Second acetone—one to one-half hour
Third acetone (with copper sulfate or sodium sulfate)—one and one-half hours
First xylene—one-half hour
Second xylene—one-half hour
Paraffin—twelve hours

B. Dissolving the paraffin, hydration of the block and section with the freezing microtome. For the second stage, the following procedure is advised:

Paraffin blocks are left for three hours in a 56 C. thermostat
First xylene—five hours
Second xylene—twelve hours
First acetone (with copper sulfate or sodium sulfate)—five hours
Second acetone—twelve hours, and then
Distilled water

Frozen sections are then cut 120 microns thick and received in distilled water.

It should be noted that, as a preliminary orientation, a fairly good picture of blood vessels can often be obtained in frozen sections stained with dilute acid fuchsin (15 to 20 drops of 1 per cent acid fuchsin to 100 cc. of distilled water) for twelve hours without previous acidification and without differentiation.

The importance of good fixation in a 10 per cent solution of formaldehyde should be stressed. When, for instance, the brain is fixed in toto and the solution of formaldehyde fails to penetrate into the deepest portion of the sulci, the blood erythrocytes in this region are not stained.

since the insufficient fixation results in decay of the erythrocytes. When material well fixed in solution of formaldehyde is available, the stain gives good results even after years of fixation.

The method when compared with the procedure of Pickworth has the following advantages: 1. It can be applied to old material. 2. Sections can be preserved in the original state for a relatively long time. 3. There are no precipitations around the vessels as often found in the Pickworth preparations; the picture therefore is clearer. 4. Other staining methods can be applied on sections from the same block.

Figures 1 and 2 illustrate the results obtained with this staining method in human and in animal material.

General Reviews

PATHOLOGY OF THE STOMACH

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In succeeding pages an attempt will be made to review the basic contributions of information concerning functional and morphologic abnormalities of the stomach. More than any other organ of the body, the stomach is influenced by a number of internal and external factors which can alter its function and structure. For this reason alone it is difficult to present this complicated subject as a review. With a desire to avoid confusion and repetitions, the selected material is segregated into sections, each section presenting the essential contributions to the subject of gastric pathology. The topics of the ten sections constituting this review are as follows: (1) the cytologic foundation of gastric pathology, (2) the physiologic foundation of gastric pathology, (3) dietary factors and the stomach, (4) chemical agents and the stomach, (5) roentgen rays and the stomach, (6) bacterial factors and the stomach, (7) endocrine factors and the stomach, (8) intracranial lesions and the stomach (9) anemias and the stomach and (10) general remarks.

CYTOLOGIC FOUNDATION OF GASTRIC PATHOLOGY

There is full agreement among cytologists that the mucosa of the normal mammalian stomach contains five well defined types of cells: (1) the tall columnar cell of the free surface and the more superficial portion of the crypts; (2) the cuboid cell of the neck of the gland; (3) the chief or peptic cell; (4) the parietal or oxyntic cell, and (5) the mucous cell of the pyloric and cardiac glands.

From the time of Heidenhain, 1870, it has been accepted by most investigators that chief cells produce pepsin and that the granules contained by these cells are the precursor of pepsin. Contributions by Langley and Sewall (1879, 1881) are to be considered as most important in elucidating this concept.

The parietal cell has absorbed the biggest share of the research, and yet it remains a subject of controversy. The main source of controversy is the question whether the secretion of the parietal cell is acid or neutral. Claude Bernard (quoted by Hoerr and Bensley), using a mix-

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ture of ferric lactate and potassium ferrocyanide, which on addition of acid precipitates prussian blue, concluded that the acid is formed on the surface of the stomach and not in the gland. Various histochemical methods, including the use of indicators, were employed in the effort to solve this question. Harvey and Bensley expressed the belief that after intravenous injection of ferric salts and ferrocyanide the prussian blue is precipitated only in moribund cells and that its presence in only a few cells is not proof that the acid is secreted by all parietal cells. Contrary to this, Macallum, Fitzgerald and Collip concluded from their results with the prussian blue reaction that the acid is produced as acid by parietal cells. Chambers, who employed the microdissection method and the injection of neutral red directly into the parietal cell, stated that the results seem to leave no doubt that the immediate secretion of the parietal cell with respect to neutral red is neutral if not alkaline. As to other conflicting results reported with the use of neutral red, one must realize that the experiments with neutral red are not entirely reliable, because the acid, if present, would be neutralized by diffusion during the interval between excision and examination. The observations with this test depend on diffusion of the dye into the cell, and it is logical to expect that the acid might equally well diffuse out at the same time. To counteract this objection involving the diffusion factor, Hoerr and Bensley applied the Altmann-Gersh freezing-drying method and obtained results which show that the parietal cells indicate an alkaline reaction and the crypts of the gland an acid reaction. Another point that is worthy of mention in connection with this discussion is an investigation by Gersh, who reported that on application of the freezing-drying method chloride was found in the lumen of the gland, along the free border of cells, but that it was absent from the cytoplasm of parietal or any other gastric cells of the mucosa.

With the introduction of new histologic methods and with the furthering of histologic studies under various experimental and pathologic conditions, new cells were described which differ in one or another way from the aforementioned well defined five cell types. Unsurpassed in his contributions to the histology of the stomach, Heidenhain reported in 1870 observing cells in the form of minute oval elements adhering to the external surface of the epithelial tube and particularly conspicuous in preparations made with bichromate solutions, in which they stained deep yellow. Cells apparently similar to those of Heidenhain were reported by Popoff, in 1897, in his studies of gastric catarrh, and by Harvey, in 1907, in his work on structural changes in the course of experimental gastroenterostomy in dogs.

Twort, in 1924, using in his studies on virus (distemper of the dog) a method of neutral red–light green staining, reported what he believed to be a hitherto undescribed type of cell. In addition to the

ordinary types of cells, his method disclosed the presence of differently stained cell with a large round or slightly oval nucleus, which usually stains with neutral red less densely than the nucleus of the chief or of the parietal cell. The cytoplasm of the new type cell stains a characteristic grass green; moreover, the cell is crowded with similar staining granules, which may be tipped with a fine point of red coloration. Though Twort has left the matter of the origin and functional significance of these cells undecided, his other points of discussion are rather important. These cells, he said, are found in such widely distinct species of animals as dog, rabbit and sheep, so they can have no connection either with the virus of distemper or with spirochetes found in the normal dog's stomach. Their distribution is patchy, their presence is independent of the phase of digestion, and they appear to be more numerous in young than in old animals.

Dawson, in 1925, investigating the effect of roentgen radiation on the mucosa of gastric pouches in dogs, noted a decided tinctorial difference of certain parietal cells, which held the iron-hematoxylin stain firmly, while others decolorized much more rapidly. The nuclei in the densely stained cells showed a well defined reticulum and a distinct nucleolus, while the nuclei of the pale cells, which he called chromophobe cells, were small, homogeneous and deeply stained. The distribution of these two types of cells seemed variable, and the difference of the observed parietal cells in staining and nuclear structure was not so pronounced in normal mucosa. Laroche and Laroche, in 1926, also reported the existence of two types of parietal cells in the fundus of the dog and stated that both types are present in the same portion of the tissue regardless of the phase of digestion.

Popoff (1939, 1940) agreed with the latter investigators concerning the presence of cells which are found in normal and in pathologic conditions and which differ in many ways from the well defined cellular types of the gastric mucosa. In his studies he was concerned primarily with the origin and function of the argentochrome cells of the gastrointestinal tract, a problem which in the opinion of Macklin and Macklin is not yet solved. Popoff's studies were made on the rabbit's stomach and on human surgical material immediately fixed in Helly's fluid, cut in serial sections and stained by his three variants of the silver method. Of his observations, the essential ones are as follows: Under normal circumstances the mature parietal cell passes through successive phases of secretory activity many times, and when finally the cell reaches the stage of functional exhaustion, it does not perish but becomes refractory, loses its response to normal and artificially applied secretory stimuli and undergoes a rearrangement or cytomorphosis, which is manifested by certain nuclear changes and a gradual accumulation and disappearance of a substance which has the property of reducing certain

metallic salts. It was thought up to now that argentaffin granules contained an orthodioxybenzol carrying a side chain in the para position. Recent studies by Jacobson, however, show that argentaffin granules contain a purine compound (probably xanthopterine or uropterine) and that in addition they contain a carbohydrate—probably a desoxypentose. In the course of this cytomorphosis the cell dedifferentiates, retracts toward the basement membrane and returns eventually to the state of a mature parietal cell. This cytomorphosis of the functionally exhausted cell was designated by Popoff as a phenomenon of functional rejuvenation. According to this view, the parietal cell is endowed with a biologic faculty of maintaining its longevity and usefulness by cyclic return, when functionally exhausted, to its primitive nonfunctional state, each return being followed by progressive redifferentiation and full recovery of its efficiency. According to Popoff, the ability of functionally exhausted parietal cells to rejuvenate makes unnecessary continuous replacement of worn-out elements by means of regeneration, and it makes understandable the discrepancies, so far unexplained in histology, between the scarcity of cellular regeneration by mitosis and the wear and tear to which gastric mucosa is subjected. In collecting opinions expressed by various investigators concerning mitosis in the parietal cell, Popoff failed to find a single trustworthy report of mitosis being found in the parietal cell. Harvey stated, "I was unable to find mitosis in the parietal cell in any of my preparations," and according to Bensley, the replacement of the gland proper presents a difficult problem, for which one does not yet know the solution. A cytomorphosis of similar morphologic character and functional significance is shown, according to Popoff, by the mucous cells of the pyloric and cardiac glands. His belief is that the new cells described by previously mentioned authors represent various phases in the cytomorphosis associated with functional rejuvenation.

The matter of cytologic reversibility invites reviewing of the essentials pertaining to the genesis of the individual constituents of the gastric mucosa. According to Kirk, undifferentiated cells of the primary embryonic epithelium differentiate directly into three definite cell types of the gastric gland, and these in turn give rise to cell generations of the same specialized types. In accordance with this view, there are no definite intermediate cell stages between the undifferentiated embryonic cells, on the one hand, and the specialized cells, on the other hand. Contrary to this and according to Lim, gastric glands in the first instance are formed of nonmucoid, red-stained cells. Later these cells become mucoid throughout the whole stomach. The next type to differentiate is the parietal (oxyntic), and at a later stage still comes the chief or peptic cell.

The matter of histogenesis is closely related to the question of behavior of various specialized cells under experimental and pathologic conditions. Cade made a significant contribution to this subject. He studied the state of mucosa at the site of a gastroenterostomy produced at the region of the fundus in dogs and cats. He noted that in the course of time the glandular space at the site of the new opening becomes cystic and that the lining cells acquire an appearance similar to that of the pyloric cells. As one moves away from the anastomosis, the cells pass through a gradual transition into ordinary cells lining the fundus. This transition, in the opinion of Cade, is effected by gradual dedifferentiation of chief cells, which is manifested by the loss of their granules and of ergastoplasm,[1] or prozymogen, and transformation into muciparous chief cells of the neck with final dedifferentiation into pyloric type cells.

Further important steps in this direction were made by Harvey, in 1907. Employing similar surgical procedures, he extended his observations for a much longer time (six and half months) than did Cade. He not only corroborated cytomorphosis of dedifferentiation, reported by Cade, but in addition found that the ferment-producing chief cells after being transformed into mucoid cells may redifferentiate or, so to speak, may reassume the morphologic and functional characters which they possessed before the operation. The process, according to Harvey, is a transformation of the cells and not a replacement. Ferguson's studies, in 1928, on the regeneration of gastric glands following removal of large areas of mucosa indicate that the repair of damage in the rabbit and that in the dog are not identical. In the rabbit the regenerated epithelium does not attempt the formation of glands, or cell specialization of any kind; and in the dog the redifferentiation takes place with the mucoid cells being differentiated first, which in turn are transformed into parietal and chief cells. Recapitulating, then, the observations reported in the literature, the generally accepted opinion concerning the ontogenetic order of cytodifferentiation is (1) primitive nonmucoid cell, (2) mucoid cell, (3) parietal or oxyntic cell and (4) chief or peptic cell, and it is believed that these cellular types dedifferentiate and disappear in the order of their specialization. As to the behavior of pyloric cells in experimentally produced pyloric pouches, the report by Lim and Dott indicates that no histologic alterations could be found in the character of the glands or of the individual cells found in the wall of the pyloric pouch.

On analyzing various reports pertaining to the subject discussed, it is apparent that while the process of dedifferentiation of chief cell

1. "Ergastoplasm" is a term introduced by Garnier, in 1900, to define a basal differentiation in chief cells. It stains more intensely by certain dyes and is best shown several hours after the beginning of digestion.

and neck cells is adequately traced, the changes observed in parietal cells under similar conditions are discussed vaguely and without any definite conclusion being reached. These reports indicate that the morphologic response of oxyntic cells to injury or to any interference with their function is of a somewhat different character. The first change that is not observed in other cells of gastric mucosa is the vacuolation of the cytoplasm in the parietal cells. Sachs reported the presence of vacuolized parietal cells, which in normal dogs were rare but in pathologic conditions quite numerous, with the vacuoles very large. Among other changes, Stintzing noted after eleven days of starvation the frequent presence of vacuoles in the parietal cells, and Harvey reported that after gastroenterostomy in the dog many parietal cells enlarge and become vacuolated.

Popoff, working on rats and investigating the effect of diet 84 of Pappenheimer and Larimore, which is deficient in the character of its protein, in vitamin A and in various mineral constitutents and limited in vitamin B, has observed, and this only in parietal cells, vacuolar changes similar to those reported by the previous investigators. Every fourth day a rat kept on the said diet was killed, and the entire gastrointestinal tract was examined in sections cut in series and stained by his silver methods. He noted a hydropic change and vacuolation of the parietal cells in the animals killed on the eighth, sixteenth, twenty-fourth, thirty-second, fortieth and forty-eighth days, while the animals killed on the fourth, twelfth, twentieth, twenty-eight, thirty-sixth and forty-fourth days failed to show any evidence of similar changes. These cyclic vacuolar changes reappearing every eighth day are observed mostly in the middle zone of the functional mucosa and only in certain parietal cells, while the rest of the parietal cells in the same section appear free from these changes. He interprets these findings as indicating that not all parietal cells are equally sensitive to the dietary deficiency tested by him and that this deficiency affects or injures only the functionally mature parietal cells. The hydropic and vacuolar changes, in his opinion, are of a degenerative character and have nothing to do with the dedifferentiation cytomorphosis described by Cade, Harvey and others, or with the rejuvenation cytomorphosis earlier reported by him.

The next histologic problem which is in line with the presented reviews of cytomorphosis is that of heterotopia or metaplasia of the gastric mucosa. Since Kupffer first described islets of intestinal epithelium in the stomach, in 1883, there have been numerous reports in the literature with valuable information, as well as controversies, as to the mode of their origin. Giving due consideration to early reports in the literature on this subject, one nevertheless must understand that in the days when the histologic foundation was not yet laid down properly

and when so little was known of the influence of diet and other factors on the state of the gastric mucosa, the concept of congenital faults presented by Conheim, in 1877, naturally dominated the minds of the majority of reporters. With the progress of knowledge and the accumulation of material covering all sorts of diseases, it became apparent that metaplasia is found in a number of different and unrelated conditions, the extent varying from the replacement of a few scattered islets to the replacement of the entire gastric mucosa by intestinal epithelium.

It is said (Magnus) that the criteria for the identification of intestinal epithelium are as follows: (1) the surface epithelium is columnar, with a striated border and with goblet cells scattered between the columnar cells and (2) the crypts of Lieberkühn are lined by columnar epithelium with occasional goblet cells, argentaffin cells and Paneth cells at their base. On taking into consideration the reviews presented concerning histogenesis and the mode of cytomorphosis as shown by various specialized cells of the gastric mucosa, such criteria must be considered as too strict. The process of dedifferentiation and of proper or faulty redifferentiation implies gradual cytomorphosis which requires a long time. This explains the reported discrepancies concerning varieties in cellular types and the extent of metaplasia found. Moreover, it is apparent that the type and extent of metaplasia and the failure of finding it at all depend on the amount of gastric mucosa examined in each case. In a series of 73 unselected autopsies Chuma found areas of intestinal epithelium in 20 per cent, while, of 40 cases in which surgical material was examined, he found it in 70 per cent. Of the 100 specimens of stomach removed for simple or malignant ulcers, or for duodenal ulcer, Magnus found intestinal epithelium in 59 per cent. Metaplasia appears to be very common in carcinoma of the stomach, and Konjetzny found it in 100 per cent of his specimens removed for this ailment.

Evidence brought forward by various investigators is in support of the view that the presence of intestinal epithelium in the stomach does not represent congenital fault but reflects the morphologic instability of specialized gastric cells, which, being easily injured by a variety of intrinsic and extrinsic factors, undergo cytomorphosis of dedifferentiation with subsequent formation of all kinds of cellular anomalies, known in pathology as metaplasia. In accordance with this view, which conforms with the old dictum by Sachs, in 1887, metaplasia or heterotopia never occurs in the normal stomach. The well established instability of the specialized cells of the gastric mucosa is abundantly demonstrated by reports of numerous investigators, and bears an important relation to a number of pathologic and clinical problems This will be reconsidered and supplemented with additional information concerning other aspects of morphologic pathology in succeeding sections.

PHYSIOLOGIC FOUNDATION OF GASTRIC PATHOLOGY

This review will be limited to those points in physiology which are essential for proper interpretation of the functional pathology of the stomach. The first stimulus to the secretion of gastric juice is nervous in origin and is dependent on the gratification of the appetite and the pleasure of taking food. This psychic secretion can be elicited only if the vagus nerve is intact, and it cannot, therefore, be produced after vagotomy, during psychic inhibition of the vagus nerve or under the influence of atropine. Initiated as appetite juice, it continues as a secretion activated by the stimulation exerted by the food on the gastric mucosa, and, as was shown by Popielski, it still occurs after all nerves proceeding to the stomach are cut. It is believed that this gastric or chemical phase of secretion is effected through the action of a hormone described by Edkins, in 1906, and called gastrin. The conclusion which Edkins drew from his experiments is to the effect that half-digested products of the earlier stages of gastric digestion act on the mucous membrane of the stomach in such a way that a hormone is produced, which is then carried by the blood to the cells of the gastric glands, on which it directly develops an exciting effect. These observations of Edkins have been confirmed, and they explain very simply how gastric secretion is maintained after the cessation of the secretion of the appetite juice. By such a mechanism, gastric juice would continue to be secreted so long as any half-digested food remained in the stomach.

It has been shown, however, that more or less continuous secretion of gastric juice in the absence of food and evident psychic factors is a normal phenomenon both in man and in animals. Complete rest of the gastric glands is an exceptional state in healthy persons and does not occur for a long period, even in prolonged fasting. The continuous secretion is true gastric juice, containing hydrochloric acid and pepsin. According to Carlson, the term "continuous secretion" should be understood in the sense that the secretion is usually not uniform in rate but the periodicity of the continuous secretion is less marked than the periodicity of the motor phenomena in the empty stomach. Boldyreff, using dogs with gastric and intestinal fistulas, found that after twenty-four hours of fasting the gastric glands begin to secrete abundant juice periodically and that after three to four days of fasting the secretion becomes continuous and so abundant as to inhibit the motility of the fasting stomach. Carlson observed in man fasting for five, eight and fifteen days no significant change in the rate of continuous secretion. The mechanism or the cause of the continuous secretion is unknown.

While the dependence of gastric secretion on the nervous and hormonal elements is firmly established, the mode of action of the latter still remains a matter of debate. The approach to elucidate the effect of the

vagus nerve covers all sorts of experiments in which the action of this nerve is stimulated or paralyzed by various physical, chemical and surgical means. In the majority of instances, the experiments have been carried out with the purpose of investigating the physiologic character of the secretion and the chemical composition. Only a few reports deal with the relation of the vagus nerve to the morphologic state of the specialized secretory cells of the gastric mucosa. Lion and Theohari, by killing dogs on the ninth, thirty-fifth and forty-sixth hours after vagotomy and examining the mucosa between the fifth and eighth hours after feeding, found chief cells to be voluminous and absolutely clear, with no granulations, which are present normally. The parietal cells were found clear around their nuclei, with the granulations being tossed to their peripheries. Cade's findings in dogs observed two months after vagotomy and six hours after feeding are similar; the parietal cells become less granular and the chief cells show no basal differentiation and are less chromophilic. Of particular value are the studies by Thomsen. On dividing both vagus nerves just below the diaphragm and making the histologic examinations at the time when the dog presented a condition of absolute achylia, he found the following picture: Instead of an expected resting stage, the morphologic state found was that which is usually interpreted as corresponding to full activity, with no signs of degeneration. The cells lay distended, with the secretory tubules opened, and this picture created the impression that the cells were unable to evacuate their contents, or, as Thomsen put it, there occurred cell obstipation. The cells merely ceased to function on account of their mechanism of innervation having run down. These findings led Thomsen to the clinically significant conclusion that the impaired mechanism of innervation can reduce the function of the gland cells for a length of time without cells becoming destroyed, or, as he said, "degeneration of the gland cells means achylia, but achylia does not at all mean degeneration of the cells."

The morphologic background presented serves thus to explain the secretory response which follows vagotomy. According to the review by McSwiney, in 1931, which covers the subject of innervation of the stomach, the effect of section of the nerve is divided into two stages: an initial inhibition and paresis, followed after an interval by a return of normal function. Section of the nerve produces only one permanent symptom, i. e., a decrease in initial emptying time. The immediate effects of vagotomy, splanchnotomy and denervation of the stomach are similar, namely, retardation of function until the intrinsic nervous mechanism assumes control of the denervated stomach. To this must be added an observation by Keeton, Koch and Luckhardt, who found that doubly vagotomized animals give excellent secretion following injection of gastrin and histamine.

It is agreed that under normal circumstances both direct and indirect stimulation of the vagus nerve through insulin hypoglycemia stimulate both the acid and the pepsin production, but the effect of any other secretory stimulant is not yet fully understood. Popielski found that the effect of histamine on the gastric secretion is independent of vagotomy, and it was his opinion that histamine acts directly on the glands. The theory that finds favor among physiologists (Babkin; Vineberg and Babkin; Gilman and Cowgill; Webster; Bowie and Vineberg; Toby; Ihre; Friedman) is that the parietal or acid-producing cells are practically the only cells of the gastric glands that are stimulated by histamine. In accordance with this view, the histamine does not stimulate the secretion of pepsin but rather produces a large flow of acid, which serves to wash out from the tubules pepsin which has accumulated therein. This concept implies that when vagal tonus is not altered, variations in pepsin eliminated depend on the rate of flow of gastric juice, and consequently, when the flow of gastric juice is accelerated, the pepsin is washed out and its concentration temporarily rises. This theory is not shared by a number of other investigators. To test out the correctness of this theory, Rivers, Osterberg and Vanzant carried out experiments in the following manner: In 71 persons, they injected a dose of histamine to wash out any pepsin which might be in the tubules, and then, before any more of the ferment could be accumulated, they gave another injection of histamine. The quantity of pepsin secreted after the second injection not only was lessened as it would have been if there had been only a washing-out process but was slightly increased. From this they concluded that histamine is a powerful stimulant to the cells which secrete pepsin. Another argument against the washing-out theory, brought up by Osterberg, Vanzant, Alvarez and Rivers, is that occasionally one observed independence of the activities of the parietal and chief cells, with high concentration of pepsin in the juices, which contained no free acid. The criticism frequently made by physiologists of clinical investigators for their technical weakness may be sound, but the fact remains that unusual variations and dissociation of the components of the gastric juice do occur, and, as Babkin said, a full understanding of the secretory mechanism of the gastric glands can be finally achieved only through the closest cooperation between the laboratory and the clinic. One point of agreement appears to be that the gastric secretion of mucus is influenced neither by vagal stimulation through insulin hypoglycemia nor by histamine (Ihre).

Returning to other fundamentals of gastric secretion, the next subject to be reviewed is the acidity of the stomach. The acidity of normal gastric juice was demonstrated probably in the first instance by Réaumur, in 1752, and later by Spallanzani, in 1780 (quoted by Oliver and Wilkinson), but it was not until 1824 that Prout showed that this was

due to hydrochloric acid. A question that has puzzled physiologists since that time concerns the mechanism by which hydrochloric acid is secreted. Giving due consideration to the arguments advanced by cytologists, the majority of physiologists believe that acid is not really secreted by the parietal cells but is formed after secretion, probably from the chlorine secreted by the cells into the fovea as some weak chloride, i. e., ammonium chloride, or it may be as an ester. This weak chloride, shortly after its secretion, undergoes hydrolytic or other dissociation, during which free hydrochloric acid is liberated, and ammonia or some other weak base is reabsorbed by the gland cells, but the hydrochloric acid is left behind because the cells are impervious to it. Using a permanent gastric fistula and applying Sorensen's electrometric method, Thomsen found that under normal conditions the p_H of the dog's gastric content is 1.00, which is close to the lowest values of healthy young persons.

The classic Heidenhain-Pavlov theory is that hydrochloric acid is secreted at a constant high concentration in both normal and pathologic conditions and that this so-called primary acidity undergoes secondary reduction through neutralization and dilution by mucus. As the primary acidity is an inaccessible value, the actual acidity and its variations become subjects of clinical importance. According to Hollander, the following factors regulating acidity of the stomach are to be considered:

1. Extragastric: (*a*) dilution by the test meal; (*b*) dilution and neutralization by saliva; (*c*) dilution and neutralization by regurgitated duodenal fluid.

2. Intragastric: (*a*) dilution and neutralization by mucus secreted from the surface epithelium; (*b*) dilution by peptic secretion; (*c*) reabsorption of hydrochloric acid already secreted by the gastric mucosa; (*d*) possible variations in the composition of the parietal secretion itself.

In the opinion of Hollander, there can be little doubt that the observed variations in acidity are due primarily to neutralization by buffer substances (i. e., protein, phosphate and bicarbonate) and to dilution by the fluids which contain them. However, Hollander left unanswered the questions as to which are those fluids and as to whether there is one which serves to a greater degree than any other in the buffer capacity. Pavlov's concept of neutralization by gastric mucus as being the most important is not shared by the majority of physiologists. Normally, mucus makes up at most 10 per cent of the volume of gastric juice; its bicarbonate content in man has been found to be 3 to 11 milliequivalents per liter, and its neutralizing and acid-binding capacity is insignificant (Ihre). Boldyreff's theory of duodenal regurgitation has been undermined by a number of investigators, who pointed out that the acidity of the gastric juice falls even when duodenal regurgi-

tation is prevented (Maclean and Griffiths; McCann; Shay, Katz and Schloss). Unable to find indications of neutralizing and diluting processes and applying a physicochemical concept, Teorell offered a diffusion theory, according to which the hydrochloric acid diffuses steadily out of the stomach contents into the mucosa cells or the blood and is exchanged against alkali chlorides (neutral chlorides) which diffuse the other way. In his opinion, this back diffusion of the H ions is a dominating mechanism in regulating the acidity of the gastric juice.

Of Hollander's regulating factors, that of possible variation in the secretion of the parietal cells itself deserves special consideration. In this connection the two old and conflicting concepts of Pavlov and Rosemann must be recalled. According to Pavlov, this secretion as poured out by its parent cells is at an acidity level which is relatively invariant; contrary to this, Rosemann's concept is that the acidity of the parietal cells is not constant and undergoes variation in a magnitude parallel with the rate of formation of the secretion. Taking into consideration the knowledge concerning the morphologic instability of the parietal cells, their particular sensibility to damage and their functional life cycle, one has little ground for believing that the constancy in the functional efficiency of these cells may be taken for granted, especially if the stomach is diseased. Furthermore, the remarkable contribution by Davenport concerning the mechanism of the formation of hydrochloric acid brings the whole matter of the regulation of gastric acidity back to the parietal cells. The foundation for Davenport's investigations was laid down, in 1931 and 1932, by Brinkman and Margaria, who discovered the existence of an enzyme which, it was found later, is present in the erythrocytes and not in the plasma. This enzyme, carbonic anhydrase, catalyzes hydration and dehydration of carbon dioxide ($CO_2 + H_2O \rightleftarrows H_2CO_3$) and plays an important role in the exchange between the tissues, plasma and corpuscles during the uptake of carbon dioxide and the unloading of oxygen (Lambie).

Davenport and Fisher and later Davenport demonstrated in rats, cats and dogs that as compared with other cells of the gastric mucosa the parietal cells show a high concentration of carbonic anhydrase. They expressed the belief that the enzyme is a part of the mechanism which secretes hydrochloric acid. According to Davenport, carbon dioxide is produced within the cells from the combustion of metabolites and is then rapidly hydrated to carbonic acid by the action of carbonic anhydrase. The carbonic acid ionizes, giving H^+ and HCO_3^- ions. By some unknown means, the H ions are concentrated and secreted, while the HCO_3 ions pass into the blood to replace the chloride ions which accompany the H ions in the secretion. If carbonic anhydrase in the gastric mucosa were inhibited, the chain of reaction would be interrupted, and the mucosa would be unable to secrete acid. This argument was

supported in further investigations by Davenport, which showed that carbonic anhydrase can be inhibited by thyocyanate ions, with consequent inhibition of acid secretion, and that the degree of inhibition of the secretion is that which is to be predicted from a knowledge of the nature of the inhibition of carbonic anhydrase by thiocyanate.

The inhibition apparently depends on the formation of an inactive dissociable compound between a mol of thyocyanate and a mol of enzyme. Booth, in 1938, found an active inhibitor of carbonic anhydrase in the serum of the pig, sheep, cat and rat, and though he failed to find a similar inhibitor in the blood of man or in that of the monkey, duck and pigeon, he stated that there is no reason not to believe that the inhibitor exists in the blood in vivo. In speaking of inhibition of the gastric secretion one must recall that this phenomenon has been known for many years, starting with the observation that a fatty meal inhibited such secretion as well as gastric motility. Kosaka and Lim, in 1930, and Ivy and Gray, in 1937, showed that this inhibition was accomplished by the production of a hormone (chalone or enterogastrone) in the mucosa of the upper part of the small bowel when this was in contract with fat. This principle when injected intravenously in dogs induced marked inhibition of gastric secretion, both as to volume and as to acid content. Brunschwig and associates reported the presence of a secretory depressant in the gastric juice of patients with pernicious anemia and in the achlorhydric gastric juice of patients with carcinoma of the stomach. Friedman and associates obtained from extracts of the urine of normal persons substances which when given intravenously inhibited gastric secretion stimulated by histamine. They stated that they did not yet know the nature of the substances, nor what organ or mechanism of the body is responsible for their elaboration.

In considering Davenport's theory one finds that there remains unanswered the question of the finding and explaining of a mechanism that is capable of concentrating the acid against a diffusion gradient and providing the necessary energy. The concept of back diffusion of the bicarbonate ions into the blood, as presented by Davenport, aids in explaining the observation that during acid secretion the bicarbonate content of the gastric venous blood is raised (Bulger, Allen and Harrison; Dodds and McIntosh).

Though both carbon dioxide and chloride are considered by all as being involved in the course of acid formation, there seems to be little unanimity of opinion as to how far their changes in the blood can influence the nature of gastric secretion. Delhougne reported that hyperventilation produces lowering of the acidity, while inhalation of carbon dioxide, according to Bakaltschuk, increases the acidity. Brown and Vineberg, working on dogs, concluded that diminution of total carbon dioxide (dissolved and combined) in the blood lowers both the volume

and the concentration of hydrochloric acid in the gastric secretion. In line with this, Apperly and Crabtree stated that the total amount of hydrochloric acid secreted by the stomach is proportional to the amount of carbon dioxide dissolved in the plasma, whereas the concentration of the acid is determined by the plasma bicarbonate, and in a subsequent paper these authors stated that a recent survey relating to this subject confirmed the foregoing assertion. Contrary to this, Taylor and Michael, using a Pavlov pouch, were unable to demonstrate any correlation between the concentration of carbon dioxide in the blood and the production of hydrochloric acid.

The problem of chloride and hydrochloric acid production was studied by a number of investigators with the following results: Neither sodium chloride deficiency in the diet nor continuous withdrawal of the gastric content, with subsequent diminution in blood chloride, affects the acidity or the chloride concentration of the gastric juice (Dragstedt and Ellis; Katsch and Mellinghoff; Mellinghoff and Heuschert; McCance; Soley, Lagen and Lockhart; Lyall and Nicol). These findings are in conformity with the opinion expressed by Carlson, in 1923, that in chloride starvation the gastric juice can produce hydrochloric acid until the secretion itself ceases from general cachexia. The total chloride concentration in the gastric juice may exceed the concentration of chloride in the blood, and Gilman and Cowgill suggested a one way permeability of the gastric mucosa to the chloride ions in explanation of the concentration achieved and retained. Toby expressed the belief that the main function of the parietal cells is to concentrate chloride to a level above that of the blood and to convert it into hydrochloric acid. This concept conflicts with the already cited work by Gersh, who failed to find chloride in parietal or any other cells of the gastric mucosa.

DIETARY FACTORS AND THE STOMACH

In considering the review so far presented, it is obvious that in many instances the picture faced by the pathologist is merely the cumulative result of disturbances caused by various sorts of injury, and for this reason an analysis of the effects produced by certain injurious agents is necessary. The first injurious agent to be discussed is diet. Initiated by the study of disease, dietary experiments have been advanced by modern science to an unrivaled success. It had been known for many years that gastric disturbances were a part of the syndromes associated with such diseases as rickets, scurvy, beriberi, pellagra and all sorts of malnutrition and anemia. Eijkman and Grijns, in 1896, presented the first scientific evidence of the existence of accessory food factors by demonstrating experimental polyneuritis in pigeons fed on polished rice. This was followed by the work of Hopkins, in 1906, who described accessory food factors. As a result the whole subject of nutrition became

a matter of experiment, the result of which as related to the stomach will be reviewed in succeeding pages.

Hyperplastic and ulcerative lesions in the stomach of the rat were first described by Singer, in 1913, who believed them to be infectious in origin. He reported their presence because Fibiger was at that time working on the relation of Spiroptera to gastric carcinoma in the rat. Pappenheimer and Larimore, in 1924, demonstrated for the first time that the lesions were in some way related to a deficiency in the diet. The diet they employed was deficient in the character of its proteins, in vitamin A content, in various mineral constituents and possibly to a limited degree in vitamin B. In 85 per cent of their rats kept on this diet for from twenty-seven to thirty-five days, they showed gastric lesions. These lesions were observed in the rumen (forestomach) and were manifested by thickening of the squamous cell lining, ulcerations and papillary hyperplasia. In their gross and microscopic pictures they showed a minute correspondence to the changes reported by Fibiger (1913, 1914, 1920) in his rats, which apparently were kept on an inadequate diet. Similar but more detailed studies were made by Howes and Vivier (1936), who, using a modification of a diet described by Pappenheimer and Larimore, observed gastric lesions in 9 of 10 young rats. The hyperplastic and ulcerative changes were observed in both the rumen and the glandular portion of the stomach. No evidence of gastritis similar to that seen in association with human peptic ulcer was found. They reported that whole yeast added to the diet protected against the development of hyperplastic and ulcerative lesions, and consequently their conclusion was that the lesions were the result of inadequacy in the diet. After reviewing results of others and his own, Cramer came to the conclusion that nematodes (Gongylonema neoplasticum) cannot be held responsible for the epithelial hyperplasia observed in the rat's forestomach and that further studies are needed to determine the exact nature of the factors causing development of gastric papillomatosis. In all reports dealing with this subject the attention is centered around the relation of the dietary deficiency to hyperplasia and neoplasia, and no particulars are given as to the behavior of the specialized cells of the gastric glands under such experimental conditions. Popoff, in the studies which were discussed in the section dealing with the cytologic foundation of gastric pathology, gave attention primarily to the effect of the same diet 84 of Pappenheimer and Larimore on the specialized cells of the gastric glands. From these studies Popoff concluded that the dietary deficiency tested by him affects only functionally mature parietal cells, causing hydropic and vacuolar degeneration. This conclusion is supported by the observation that, cyclic in character, this degeneration reappears every eighth day and that it affects only certain parietal cells in the middle zone of the functional mucosa, while the rest of the parietal

cells in the same section remain intact. It appears, then, that cellular infiltration and hyperplastic and ulcerative changes cannot be properly interpreted without taking into consideration the damage to the specialized cells and the effect of this on the state of the entire gastric mucosa.

The relation of vitamin B deficiencies to congestive, hemorrhagic, ulcerative, atrophic, hypertrophic and papillomatous changes in the stomach is reported by a number of investigators (McCarrison; Findlay; Sure, Thatcher and Walker; Dalldorf and Kellogg). To the work reported by these authors must be added observations on the relation of vitamin B deficiencies to the decrease in gastrointestinal motility and tonicity and to the degenerative changes in the nerves (Rose; Elsom; Vorhaus, Williams and Waterman; Sparks and Collins; Strauss; Theobald; Russell; Jolliffe, Colbert and Joffe; Holman). Cowgill and Gilman studied the volume, acidity and proteolytic power of gastric secretion in response to histamine in dogs with Pavlov and Heidenhain pouches and found that on a diet deficient mainly in vitamin B_1 there was diminution of gastric secretion with occasional achlorhydria.

Though vitamin C deficiency is not definitely related to the production of gastric lesions, decrease in vitamin C values has been reported in association with gastric hemorrhages, ulcer and carcinoma by a number of investigators (Ingalls and Warren; Lazarus; Chamberlin and Perkin; Holman). To stress the influence of vitamin C, the pioneering work by Hojer (1924) ought to be recalled. He found that ascorbic acid is intimately connected with the synthesis and maintenance of the interstitial supporting material, which provides the framework for healing. His work formed the basis for valuable investigations concerning the influence of vitamin C on the healing of the wounds and the underlying pathologic changes in scurvy (Lanman and Ingalls; Wolbach; Taffel and Harvey).

In the newborn, hemorrhages are found to be responsible for a great percentage of the pathologic conditions characteristic of that stage of life. They are particularly apt to occur in the alimentary tract, as many as 69 per cent of all infants showing their occurrence in that organ. In hemorrhagic disease of the newborn the most striking pathologic change is the prolonged coagulation time, with a deficiency of prothrombin in the blood as the most probable cause. The prothrombin deficiency in this disease was reported as early as 1912, by Whipple. Dam and Schonheyder, in 1934 and 1936, demonstrated the existence of a specific factor, designated as vitamin K, the role of which is to regulate the prothrombin content of the blood. A lack of vitamin K causes coagulation disorder, and it is a definitely established factor in hemorrhagic disease of the newborn. Ulcers of the stomach and duodenum are demonstrated in

about half of the patients who die following melena, and it is worthy of note that in his review of hemorrhagic disease of the newborn, in 1935, Salomonsen stated that a number of circumstances justify the inclusion of melena in the group of hemorrhagic deficiency diseases. In man, vitamin K is probably of endogenous origin, being synthesized by bacteria in the alimentary tract. Salomonsen suggested that the lack of vitamin K in the newborn may be due to deficient production, because of sterility of the alimentary tract, or to deficient absorption. A criticism that may be raised against the observations dealing with vitamins and the stomach is that while they furnish valuable general and statistical information, most of them are outside the field of properly controlled experiments.

In addition to the dietary factors just discussed, a brief reference must be made to the gastric changes resulting from dietary deprivation of protein. Weech and Paige, after reviewing this subject, presented their own observations on the effect of a diet deficient in protein but adequately supplied with vitamins. Of their 22 dogs kept on such a diet, 36 per cent exhibited true peptic ulcer and 23 per cent superficial erosion of the gastric and duodenal mucosa, while 41 per cent showed neither ulcer nor erosions. Since accessory data concerning acidity, enzymes and motility were not obtained, the authors found it proper to withhold their opinion concerning the nature of the relation between peptic ulcer and a diet deficient in protein. Impairment of the liver is closely related to various disturbances, including a protein deficiency, and there are references in the literature concerning a possible role that this organ may play in the genesis of gastric ulcer. It is reported that many types of hepatic injury (Eck's fistula, ligation of the common duct, bile fistula, hepatoxin) are followed frequently by formation of peptic ulcers, usually of duodenal location (Crandall and Ivy; Hawkins and Whipple). The exact mechanism involved in the formation of such ulcerations remains unknown.

CHEMICAL AGENTS AND THE STOMACH

The next point of inquest which emerges from the reviews of the "etiologic complex" is the gastric injury produced by certain chemical agents. In this presentation the priority must be given to alcohol, which is of interest to both the toxicologist and the student of dietary deficiencies.

Since Shattuck, in 1928, called attention to the occurrence of beriberi in persons with chronic alcoholism, a number of investigators have pointed out that avitaminosis plays a much more important role than the direct toxic action of alcohol and that the vitamin B_1 deficiency actually is the primary cause of the polyneuritis and other symptoms in the alcohol addict (Wechsler; Minot, Strauss and Cobb; Goodhart and

Jolliffe). Villaret, Moutier, Justin-Besançon and Klotz reported 50 cases of alcoholic polyneuritis and found that following the histamine test there was achlorhydria in 33 of them, hypochlorhydria in 12 and normal acidity in 5. It is apparent that the incidence of achlorhydria is far higher in patients with alcoholism and polyneuritis than in those with uncomplicated alcoholism, a suggestion first made by Minot, Strauss and Cobb, in 1933. Seymour, Spies and Payne extended similar investigations by making repeated histamine tests in alcoholic addicts with no clinical evidence of vitamin deficiency and no laboratory evidence of anemia. On repeating the histamine test following enforced abstinence from alcohol, they found that of 40 persons who were achlorhydric in response to the first histamine test, 7 yielded free hydrochloric acid. This point is significant not only in its application to the interpretation of reports based on single tests but also in its suggestion of the relationship of the ingestion of large quantities of alcohol to the secretion of acid. It appears, therefore, that as far as gastric secretion is concerned, the toxic action of alcohol cannot be disregarded, and in this connection the fine experiments and their interpretation presented by Thomsen before the Hunterian Society of London, in 1933, are very worthy of mentioning. In his experiments on dogs the alcohol was introduced into the lavage-evacuated stomach and then after five to ten minutes was evacuated from the stomach. The experiments lasted for one and a half years, with the animal receiving alcohol every other day and later two times a week, and the stomach contents and specimens of tissue being obtained for examination. Analysis of the stomach contents showed that following the introduction of alcohol there was increase in volume and acidity, with return to normal after suspension of the administration of alcohol and with diminution of volume and acidity when administrations of alcohol were made more frequently. The first phase of alcoholic gastritis excites the secretory function of the parenchyma, and it is not inflammation alone that acts on the peripheral nervous apparatus or on the glandular cells themselves. The principal cause is the direct irritation of the parenchyma by the alcohol. After one and a half years of alcoholization of the mucosa, the secretory activity of the glandular cells had fallen down to a very low level, and it was expected that glandular cells would be found altered, poor in secretion and partly destroyed. A different picture, however, was observed. On numerous sections obtained from different preparations the glandular tubes were neither cystic nor atrophic nor destroyed. They were simply a little more distant from each other, owing to thickening of the interstitial tissue. On comparing the histologic picture of the doubly vagotomized stomach with that of alcoholic gastritis, a striking similarity is found. The secretory cells are in a state of obstipation, and their appearance corresponds to the stage of activity. Though remaining undamaged, the

parietal cells appear to be unable to discharge their secretory content on account of paralysis of the intercellular nerve terminations. If alcoholization is continued, the inflammation progresses, and the glandular tubes undergo cystic and other changes, which finally end in atrophy of the functional mucosa. These and other similar reports in the literature indicate that in interpreting functional and organic disturbances caused by alcoholism, both the direct toxic effect of the alcohol and the vitamin B_1 deficiency must be taken into consideration.

After the work of Dale and Richards on the vasodilator action of histamine and of some other substances became known, in 1918, the interest was centered around histamine and its possible relation to a number of clinical problems. The two properties of histamine—one to produce transient vasoconstriction in the arterioles and more delayed and prolonged vasodilatation of the capillaries, and the other to activate the secretion of hydrochloric acid—suggest theoretically that histamine may be a factor in the production of certain gastric lesions, especially peptic ulcer. Büchner, Siebert and Molloy produced lesions in rats by increasing gastric secretion in them with histamine. It must be remembered that the character of the diet given to these animals was not mentioned and that it was said that alternate days of fasting increased the incidence of the lesions and that when the rats ate feces no lesions resulted in spite of the injection of histamine. Bürkle-de la Camp performed similar experiments and obtained the same results. Dodds reported that large doses of histamine never produced gastric lesions in rabbits. The discrepancies reported suggest that other factors besides hyperacidity caused by histamine are needed for the production of hyperplastic and ulcerative lesions in the stomach.

Cinchophen is a substance which appears to possess the property of damaging gastric mucosa, independent of the state of nutrition. This drug has been used in the treatment of gout since 1911. Churchill and Van Wagoner, in attempting to produce hepatic damage by oral administration in cinchophen, in 1931 and 1932, noted gastric ulcer occurring in 24 of 30 dogs used. The earliest ulcers developed in eight to ten days. In 90 per cent of the animals the ulcers were situated in the gastric pathway and were generally multiple. The major symptoms noted were anorexia, occasional vomiting, tarry stools and loss of weight.

Since that time a number of papers have been published concerning experimental production of gastric ulcers with cinchophen. The results reported indicate that, whether administered internally or parenterally, cinchophen is capable of producing gastric ulcerations. Stalker, Bollman and Mann reviewed this subject in 1937 and 1938 and stated that, according to their own experimental studies, the action of the drug is not local but occurs after absorption. The effect begins with the destruc-

tion of mucosal cells. This occurs throughout the gastric mucosa and is associated with diffuse acute hemorrhagic gastritis. There is hemorrhage from the mucosal blood vessels. The mucosal erosions progress as a result of digestion by the gastric contents and become multiple acute ulcerations. These acute lesions heal, and the gastritis disappears, but the acute ulcers in the gastric pathway, probably because of the trauma and increased digestion of tissue, progress and become chronic. Pathologically, the peptic ulcer produced by cinchophen in the dog is identical in every respect with the peptic ulcer seen in man. After administration of the drug there is no change in the level of acidity, but there is an increase in the amount of gastric secretion. This initial hypersecretion is followed by the development of a peptic ulcer and hyposecretion. Peptic ulcer developed in 95.8 per cent of their dogs following the administration of cinchophen. It appears from the literature that not all animals are equally sensitive to cinchophen. Schwartz and Simonds gave cinchophen in oil orally to cats, rabbits and guinea pigs. Superficial ulcerations were noted in the stomachs of the cats but not in those of the rabbits or of the guinea pigs. According to Stalker, Bollman and Mann, in 1937, no case had been reported in which cinchophen was indisputably the cause of peptic ulcer occurring in man. Reah, in 1932, reported a case in which a patient died after treatment with cinchophen. Necropsy revealed a cirrhotic liver and a gastric ulcer. Bloch and Rosenberg, in 1934, reporting on 8 cases, suggested the possible etiologic relationship of cinchophen to hepatic damage, multiple gastric ulcers and chronic gastritis.

A number of other chemical substances are reported as having an influence on the stomach, but the information as to their effects is of rather general character. It is reported, for instance, that anesthetic agents, such as chloroform, ether, nitrogen monoxide, ethylene, cyclopropane and divinyl oxide, prolong the emptying time of the stomach and that their effects decrease in the order of their listing (Sleeth and van Liere). Tobacco smoking as related to the stomach is considered by a number of clinicians as being harmful. However, few experimental data exist on which to base an explanation of this assertion. From the experimental studies by Schnedorf and Ivy it appears that smoking reflexly inhibits or depresses the hunger motility of the stomach. When the smoking of a number of cigarets has any effect on the stomach, it tends to depress secretion and to retard evacuation. Only in occasional persons does smoking tend to cause significant gastric retention and increase of acidity. In view of the frequent clinical use of barbiturates, it is well to remember that, as shown by Coffey, Coppanyi and Linegar, these drugs produce a reduction of the gastric and pancreatic secretions with a prompt return to normal flow after hypnotic doses and a delayed return after anesthetic doses.

ROENTGEN RADIATION AND THE STOMACH

The interest centered around the roentgen ray is a good reason for including in these reviews a brief discussion of the effect of roentgen radiation on the gastric mucosa. Regaud, Nogier and Lacassagne after giving dogs five successive doses of roentgen radiation during a period of fifteen months found practically complete obliteration of the chief cells, the parietal cells still being present. Warren and Whipple indicated that the small intestine is apparently much more susceptible to injury by roentgen radiation than is the gastric mucosa. Case and Warthin, in their report on the effects of such radiation on the liver, noted marked atrophy of the gastric glands with mucoid degeneration and vacuolation.

Of particular interest is the contribution by Dawson, who gave a good account of histologic changes in the gastric mucosa of dogs following irradiation. His work was entered on with a hope that some clue as to the site and manner of the formation of hydrochloric acid might be obtained by studying the roentgen ray–treated Pavlov pouch, which manifests such striking diminution in acid production. Hyperemia with concurrent petechial and ecchymotic hemorrhages was considered as a main factor, but, as he said, it is difficult to determine which is injured primarily, the vascular supply or the gastric epithelium. On giving a total dose of 205 per cent of a dog's erythema dose, the following changes are found in the mucosa of the pouch: superficial cells and the cells of the neck have disappeared, and in many cases the injury has extended to the chief and the parietal cells in the superficial one third of the mucosa. The chief cells in this zone usually undergo lysis, and the pepsin granules are set free in the lumen of the injured glands. The parietal cells, on the other hand, seldom suffer lysis but retain their characteristic form and appearance. According to Dawson, the probable order of injury is: neck cells, mucous cells, chief cells and parietal cells.

On discontinuing the use of radiation and following a recovery of function the mucosa of the pouches differed from the normal in but two important respects, i. e., thickness and the staining of the parietal cells. The difference in the staining characters of the nucleus and cytoplasm of the parietal cells has been discussed already, in the section dealing with the cytology of the stomach. A significant feature of roentgen ray injury pointed out by Dawson is that in none of the pouches in which the production of hydrochloric acid was abolished by roentgen radiation were all parietal cells destroyed, and in view of this he concluded that persisting parietal cells are simply unable to produce hydrochloric acid. In this connection it must be mentioned that, according to Popoff, only the functionally mature parietal cells are sensitive to injury; the parietal cells undergoing the rejuvenation cycle are refractory or nonsensitive to every chemical agent tested by him.

In 2 cases reported by Palmer and Templeton an opportunity arose to study irradiated mucosa in man. The material was studied by H. G. Wells and E. Humphreys, and in 1 case the following observations were reported: The gastric mucosa is everywhere profoundly altered, especially in the fundus; mucous cells are absent from the depths of the gastric pits as well as from the surface. The most striking changes are seen at, and just below, the base of the necks of the glands. Here the glandular pattern is distorted, and sloughing degenerated cells are mingled with living cells; the surviving cells have pale cytoplasm and vesicular nuclei, often with large nucleoli. Syncytial masses with from five to ten nuclei are seen; mitoses are few; in both chief and parietal cells degenerative changes are seen; acute and chronic inflammatory changes are noted. In agreement with others, Palmer and Templeton have observed that radiation therapy directed toward the upper two thirds of the stomach in doses totaling from 1,100 to 3,600 roentgens depresses gastric secretion.

BACTERIAL FACTORS AND THE STOMACH

As a matter of general interest, it must be mentioned first of all that normal gastric glands of man and of animals frequently contain spirochetes, the presence of which has been noted by a number of investigators. The observations made by Cowdry and Scott that spirochetes occur in the gastric glands of normal-appearing monkeys (Macacus rhesus) is of interest, because this animal is closer to man than rats, cats and dogs, in which spirochetes have been reported to occur in the same location and without any accompanying signs of illness (Bizzozero; Salomon; Regaud). The majority of the spirochetes are extracellular; still a considerable number are intracellular, and they are found always in the parietal cells and never in mucous or chief cells. According to Doenges, the incidence of spirochetes in the gastric glands of Macacus rhesus was 100 per cent in a series of 43 animals. The incidence in man was more than 43 per cent. Distinctive clinical symptoms were absent, and microscopic lesions associated with the presence of spirochetes were slight or altogether lacking.

The reliability of reports concerning the bacterial flora of the stomach depends on (1) the way the gastric content was obtained, (2) the record of actual acidity of the contents and (3) the methods of bacteriologic examination. A report by Nichols and Glenn, in which these three factors were given due consideration, offers the following information: With p_H ranging from 1.74 to 2.48 and with free hydrochloric acid, there was no bacterial growth on any medium; with p_H 4.20 and free hydrochloric acid, only a sarcina and a mold (Syringospora); with p_H 4.79 and no free hydrochloric acid, abundant bacterial growth. In general, the stomach contents with p_H above 4.20 showed moderate to abundant

bacterial growth. Direct examination by the Gram method showed gram-positive and gram-negative cocci in pairs and short chains, as well as gram-positive and gram-negative bacilli. Streptococci and micrococci (nonhemolytic) were the most frequent groups encountered in the stomach. The role of the gastric secretion in preventing passage of bacteria from the mouth to the intestine has been studied intensively by Arnold and associates, who have shown that as long as free ions are present within the gastric lumen, few viable bacteria are to be found there. Florey stressed, in addition to the protection afforded by the acid mechanism, the important role played by the mucous secretion. The bacteria are enmeshed in the mucus and rendered inert, and those which are sensitive to lysozyme are dissolved. In studies on the gastric barrier in bacillary dysentery, Felsen and Osofsky found that filtered human gastric juice is bactericidal for the Sonne-Duval (D) strain of Bacterium dysenteriae when the p_H is approximately 5.50 or less and that the infectivity of this strain of organisms may be determined primarily by the degree of acidity in the stomach at the time of the ingestion of the organisms.

Though the well known resistance of the stomach to all kinds of spontaneous and experimental infections may be attributed chiefly to the gastric acidity, other reports indicate that the stomach is equally resistant to tubercle bacilli, which can withstand the action of normal gastric juice for many hours. Delille and Poulsen reported that tubercle bacilli were found in 100 per cent of tuberculous children by means of gastric lavage, and Munro, unwilling to accept reports based on the examination of smears, was able to isolate the peccant bacillus in 26, or 8 per cent, of his 82 cases. Notwithstanding these and other equally high figures on the positive findings of tubercle bacilli in the stomach contents, gastric tuberculosis is a rare condition. In 1,400 autopsies on tuberculous subjects Crawford and Sawyer did not once find gastric involvement, whereas the incidence of tuberculous lesions of the ileum and colon reached 70 per cent. Glaubitt (quoted by Broders) reported that of 2,237 patients who died of tuberculosis, gastric tuberculosis was found in 0.37 per cent. Clagett and Walters, on reviewing the problem of gastric tuberculosis, in 1938, were able to find only 368 cases of gastric tuberculosis reported in the literature. According to Chaffin's careful analysis in 1939, a total of 211 cases had been reported to the end of 1937, with 51 unquestionable. The remarkable resistance of the stomach to tuberculosis is attributed to the following factors: (1) the effect of the gastric juice on the tubercle bacilli; (2) the scarcity of lymph follicles and lymphatics in the stomach; (3) the rapid emptying of the stomach; (4) the integrity of the gastric mucous membrane; (5) the resistance of muscular tissue to tuberculous infection. While tuberculous granulomatous lesions are rare, simple gastritis is rather common in phthisic

patients. Marfan (quoted by Broders) reported gastritis present in 18 of 27 tuberculous patients on whom autopsy was performed. Perhaps the frequency of achylia, which, according to Faber, is observed in 33 per cent of patients with pulmonary tuberculosis, may be partly due to gastritis. This type of gastritis is manifested by thickening of the gastric mucosa, by hyperemia, and microscopically by round cell infiltration of the mucous and submucous layers of the stomach. Whether this type of gastritis is due to tuberculosis or is precipitated by the concurrent malnutrition remains unknown. As in the case of tuberculosis, the gastric mucosa appears to be resistant to syphilitic infection; Eusterman among 25,000 syphilitic patients found only 87 with questionable gastric syphilis.

Clinical reports state that achylia frequently develops during and after infectious diseases, such as influenza, tonsillitis, bronchitis and especially infectious enteritis. Of 64 infants with intestinal infection, Bloch (quoted by Faber) found lowered secretion in about 50 per cent; in 23 per cent he observed complete anacidity, which persisted for weeks or up to eleven months. In adults achylia develops and sometimes persists after acute infections of the intestines, such as dysentery, typhoid and paratyphoid fevers. In pneumonia, besides achylia, there is observed a rare but typical complication, which was originally described by Dieulafoy, in 1899, under the name of *gastrite ulcéreuse pneumococcique*. According to Sanford and associates, this complication is more frequent than is indicated in the literature.

In many instances infection provokes a rise in temperature, and whether fever is caused by spontaneous infection or by intravenous injection of nucleinic acid or of inactivated Bacillus prodigiosus, it leads to a notable diminution of gastric secretion and achlorhydria (Carlson; Vanzant and Snell). It is, however, interesting to note that Brunschwig and collaborators were able, after immunizing their dogs against fever-producing factors, to observe in some of the dogs secretion of hydrochloric acid in adequate quantities though the temperature was as high as 42 C. (107.6 F.). This would indicate that besides the fever there must be other factors which are responsible for temporary inhibition of the production of hydrochloric acid.

On the whole, the mechanism of the gastric injury caused by infection is not clear, and among the few contributions on this subject the work by Thomsen (1924, 1934) deserves special consideration. On injecting diphtheria toxin and observing the dog for a month, he noted a violent reaction in the stomach. Together with gastritis and gastric retention, total achylia developed in the beginning of the second week after the injection. At the time that achylia developed, the mucosa was involved in acute hemorrhagic gastritis. According to Thomsen, the suspension of secretion developed at a stage far ahead of the hemorrhagic gastritis

and even before signs of glandular injury could be detected. Before death an inflammation extended to include the entire wall of the stomach, embracing nerve fibers and plexuses. It appears, then, that in the first phase of intoxication the achylia is due to functional inhibition of the secretory cells by the diphtheria toxin, while in the second phase the achylia is the result of destruction of the functional mucosa. The pathologic anatomy of the hemorrhagic gastritis in children who died from diphtheria was studied in detail by Nyfeldt and Vimtrup, and the results of a morphologic study of the experimental erosive gastritis produced by diphtheria toxin in cats and guinea pigs was reported by Hanke.

Phlegmonous gastritis, although rare, deserves consideration. This acute pyogenic infection of the stomach may begin as localized cellulitis, which may become either a circumscribed abscess or a diffuse phlegmon. The most common organism causing this disease is the streptococcus, and direct contact with infectious material has been assigned as a cause of gastric phlegmon. In their paper on phlegmonous gastritis Cutler and Harrison stated that Sandberg found hypoacidity in all of his cases. Stieda noted that while postoperative infection developed in 33 per cent of patients with low acidity only 2.8 per cent of patients with high acidity had complicating infection. Attempts to reproduce this disease in dogs were unsuccessful. However, Doehle is quoted by Vaughan as having produced cellulitis of the stomach by feeding dogs alcohol and later a pure culture of a hemolytic streptococcus.

In relation to this discussion, there is a question of allergy and its relation to the functional and organic diseases of the stomach. Clinical reports indicate that hypochlorhydria and achlorhydria are of frequent occurrence in allergic disease. Criep and McElroy, in studying 50 cases of asthma, hay fever, urticaria, angioneurotic edema and atrophic rhinitis, found achlorhydria in 36 per cent, complete achylia in 4 per cent and hypoacidity in 32 per cent. Rowe found achylia in 10 per cent of 80 cases of gastrointestinal allergy. Whatever may be the actual cause, achlorhydria is reported in every kind of allergic disease. Demel observed that the vascular changes in an allergic reaction and in a peptic ulcer were somewhat similar and that therefore in some instances an ulcer might be a manifestation of local anaphylaxis. He suggested the Arthus phenomenon or an urticarial wheal as the exact mechanism. Ivy and Shapiro produced lesions resembling peptic ulcers in rabbits by injecting into the gastric mucosa a foreign protein to which the animal had been previously sensitized. These results, however, could not be reproduced by Alessio and Vallone. Ehrenfeld, Brown and Sturtevant found that of 75 patients with lesions proved to be peptic ulcer only 10 per cent were allergic, and they concluded, therefore, that there is no valid reason to assume that allergy plays an important part as an etiologic factor in peptic ulcer. Kapp tried to find whether patients with gastric

disease (chronic and subacute gastritis, chronic gastroduodenitis, pernicious anemia) were sensitive to the bacterial filtrates from their own gastric flora and reported great variation in the response to intradermal tests. In the control group the difference was in severity of reaction only; furthermore, 20 per cent of the controls gave reactions with severity equal to that shown by persons with gastric disease. Giving due consideration to clinical and statistical information, one is disappointed to find in the literature so little in the way of substantial knowledge concerning bacterial factors and allergy as related to functional and organic diseases of the stomach.

ENDOCRINE FACTORS AND THE STOMACH

There are few reports in the literature concerning gastric disturbances associated with changes in the endocrine glands. Predominantly of functional character, these disturbances cannot be left without consideration. The consensus is that hyperthyroidism causes either hypochlorhydria or achlorhydria. Moll and Scott examined 50 patients with the syndrome of exophthalmic goiter and found that 44 per cent had achylia. The mechanism of achylia, according to these observers, is twofold: (1) there is associated overaction of the vagus nerve; (2) there is a direct inhibitory action of the sympathetic nerves on the secretion of hydrochloric acid, causing true achylia. Investigating the effect of thyroidin, Thomsen found that this substance diminishes the quantity of secretion while only slightly modifying the quality, and that the changes observed in the parietal cells never come to any lasting degeneration. The secretion is diminished merely as the result of a toxic action of thyroidin, and when intoxication is stopped, the gastric glands return to the normal state. This remarkable observation by Thomsen indicates, then, that the thyroidin intoxication affects or damages only the function of the parietal cells and causes achylia or hypochylia without coexisting signs of inflammation and cell degeneration.

The parathyroid glands are not associated with any definite pathologic change of the stomach. The tetany of parathyroidism has been studied in relation to its effect on gastric function. Carlson, investigating conditions of the digestive tract in this condition, has found that the motor deviations observed are in the direction of depression. Following parathyroidectomy, Keeton observed that (1) the quantity of gastric juice was lessened, or this secretion was entirely suppressed, (2) there was depression of free hydrochloric acid, and (3) while in some instances the acid content of the juice was fairly high, the peptic activity was very low.

Of all the glands of internal secretion, the pituitary gland stands out as one which can exert a direct specific influence on the stomach. In a

number of papers, dating from 1934, Dodds and his associates reported that certain gastric lesions may be produced by subcutaneous, intravenous or intramuscular injection of any posterior pituitary extract containing the pressor fraction. Extracts of the anterior and intermediate lobes, as well as the oxytocic fraction, have been shown to be inactive. If a rabbit is given a large dose of posterior pituitary extract and is killed twenty-four hours later, a definite picture is presented. The upper two thirds of the stomach is replaced by an intensely engorged hemorrhagic mass of tissue, blackened from the acid in the stomach contents. This intense reaction stops sharply at the esophageal opening and gradually fades above the incisura angularis. This sharp line of demarcation is found to correspond with the exact distribution of the oxyntic cells in the rabbit. The pyloric end and duodenum appear normal. Following capillary dilatation and engorgement, four to six hours after the injection, there is a destructive process throughout the entire acid-bearing area of the mucosa. Healing and regeneration are rapid, and after ten days the gastric mucosa appears to differ little from the normal. In approximately 24 per cent of cases there developed a typical punched-out ulcer, which microscopically showed the characteristics of a chronic peptic ulcer. From the distribution of the lesion it is clear that the presence of oxyntic cells is an essential factor in its production. The fact that animals with gastric contents of high acidity are the most susceptible, together with the demonstration that neutralization of the acidity prevents the experimental production of the lesion, has led Dodds and his associates to the following interpretation of the mechanism underlying the development of this lesion. The administration of the extract causes, first of all, partial inhibition of gastric secretion, resulting in reduction of the amount of juice produced. It is the total quantity, or volume, per unit of time, which is reduced. If still more of the extract is administered, complete inhibition of gastric secretion occurs. If the content of the stomach in which secretion has been inhibited by this means is strongly acid, severe destruction of the gastric mucosa occurs with production of the typical hemorrhagic lesion. This may heal perfectly, or it may heal irregularly and produce a chronic lesion, taking the form of an ulcer, and subacute or chronic perforation may result. A vascular factor is closely related to the maintenance of normal secretion; it follows, then, that the integrity of the gastric mucosa is also related to this factor, and, according to these investigators, a reduction in the blood flow produced by the posterior pituitary extract is the primary cause of the gastric lesion. The known vasopressive action of this extract and the production of similar gastric lesions by barium salts are added as additional evidence in support of vasoconstriction as a causative factor. Pituitary extract inhibits the secretion induced by the

taking of food, and it also inhibits the action of insulin, which is considered by these authors as the most powerful stimulant for the secretion of hydrochloric acid and pepsin. Their other observation worthy of mentioning is that pilocarpine, epinephrine and histamine administered in large doses have never given a typical pituitary lesion in the rabbit. Experiments of similar character with pitressin are reported by Nedzel, who extended his investigations along the line of Petersen's concept of the interrelationship between disease and meteorologic factors and found striking seasonable variations in the occurrence of gastric ulcers in dogs, the spring months giving the highest percentage of dogs with gastric lesions.

Related to this subject of posterior pituitary influence are the observations by Blotner on gastric secretion in diabetes insipidus. In this disease the symptoms of secretory disturbance are a great volume of gastric juice, higher acidity and an increase in pepsin and renin content. According to Blotner, the administration of solution of posterior pituitary in this disease decreases the acidity during the histamine and alcohol tests and also diminishes the volume an hour after injection of the drug.

INTRACRANIAL LESIONS AND THE STOMACH

In many experiments hypophysectomy was attempted with the purpose of obtaining additional information concerning the relation of this gland to the stomach. Results of such experiments are difficult to dissociate from the effect of the concurrent injuries to the brain, which are almost unavoidable. Perhaps it will be more appropriate to consider now the much discussed problem of the relationship between certain intracranial lesions and the state of the stomach.

Schiff, in 1867, and Brown-Séquard, in 1876, suspected lesions of the central nervous system as playing a part in the genesis of gastrointestinal ulcerations; and von Preuschen, in 1894, Wassmer, in 1909, and Zadek, in 1912, reported gastrointestinal ulceration resulting from intracranial trauma sustained at birth. Burdenko and Mogilnitzky, in 1926, were among the first to provide experimental evidence that hypothalamic injury might lead to hemorrhagic ulceration of the stomach and duodenum. Using the subtemporal approach, they produced small lesions in the base of the brain immediately behind the infundibular stalk. Gastric hemorrhages and erosions, acute ulceration and occasionally perforation with peritonitis resulted in some instances. In the animals surviving the operation by several months they found chronic cicatricial ulceration. They explained their results on the basis of destruction of a vasomotor center in the posterior part of the hypothalamus and of a metabolic center in the tuberal region.

Since Cushing's report, in 1932, on a series of patients with midbrain tumor and concurrent peptic ulcer, this subject has been given considerable attention by a number of clinical and experimental investigators. The most extensive experiments along this line were made by Keller, who used over 200 dogs in his studies on changes in the gastrointestinal tract following various surgical intracranial procedures, which in some instances were supplemented with vagotomy and sympathectomy. Watts and Fulton and also Hoff and Sheehan investigated gastric lesions following hypothalamic injuries produced experimentally in monkeys. From the publications on this subject it appears that lesions of the tuberal nuclei are associated with gastrointestinal erosions and ulcerations. The erosions in the stomach are confined to the body, both the anterior and the posterior wall. Sometimes the pyloric region is also involved, but there is no predilection for this part or for the lesser curvature of the stomach. The erosions are always multiple and hemorrhagic. They are confined to the mucosa, and in no case is the muscular coat involved. The histologic picture is one of sharply localized destruction of the mucosa and hemorrhagic exudation, with no evidence of thrombosis in the submucosal vessels underlying the lesion.

Two interpretations have been advanced in the consideration of the neurogenic factor involved in the genesis of experimentally produced lesions. One is based on a conception of sympathetic hyperactivity, and the other explains the phenomena on grounds of parasympathetic hyperactivity. The first concept implies overactivity of the sympathetic vasoconstrictor with consequent spasm, ischemia in the mucosa and digestion of the necrosed tissue by the acid secretion. Parasympathetic hyperactivity, or Cushing's concept, implies a functional release of the vagus nerve from paralysis of the antagonistic sympathetic fibers, with consequent hypersecretion, hyperchlorhydria, hypermobility and hypertonicity, especially marked in the pyloric region. The spasmodic contraction of the musculature, possibly supplemented by accompanying local spasm of the terminal blood vessels, produces small areas of ischemia or hemorrhagic infarction, leaving the overlying mucosa exposed to the digestive effect of its own hyperacid juices. Though these two concepts appear physiologically sound, the functional localization in the hypothalamus is not sufficiently clarified by the reported studies to warrant any final conclusion as to the exact mechanism involved. It is obvious that the concurrent injury of the brain, the hemorrhages and the malnutrition caused by tumors of the brain and by the operative procedures are the factors which complicate the picture and which make way for errors in interpreting the results obtained. Taking into consideration all requirements of standard preoperative and postoperative observation, Martin and Schnedorf, by electrocoagulation

with the aid of the Horsley-Clarke stereotaxic apparatus, produced the lesions in the hypothalamic area in 7 monkeys and 40 cats. Their observations follow: No bloody stool or vomitus was noted at any time from any animal; the nutritional state of each animal remained good; no significant changes in gastrointestinal activity or in secretion followed the production of the lesion; autopsy did not reveal any mucosal changes in the gastrointestinal tract which could be ascribed to the lesions. These studies by Martin and Schnedorf preclude the need for further discussions and leave the entire subject open for future research.

ANEMIA AND THE STOMACH

The reciprocal relationship between anemia and the stomach has been given a great deal of attention by both the clinician and the laboratory worker. In the interpretation of this relationship, the factors of nutritional, toxic, endocrine and congenital influences are mentioned repeatedly, and for this reason it was decided to postpone the review of this problem until the essentials of the histophysiology and of the gastric responses to the individual injurious factors had been reported.

Symptoms of gastric disease accompanied by objectively demonstrable evidence are frequently observed in association with all types of anemia. An immediate question involved by implication is this: What is the effect of a simple loss of blood on the stomach? In other words, is there any direct relationship between erythrocyte and hemoglobin values, on one hand, and the functional state of the stomach on the other? According to Apperly and Cary, when as a consequence of recent or chronic hemorrhage the erythrocyte content of the blood falls below a certain critical level, free acid disappears from the stomach. Their observations in man indicate that this level is, on an average, about one third to two thirds of the normal. That loss of blood is not the only factor is demonstrated by their further findings, which show that when severe hemorrhagic anemia is associated with peptic ulcer, pylorospasm or other gastric condition, the acidity values are far higher than those with a corresponding degree of anemia without gastric abnormality. Alvarez and Vanzant found a correlation between gastric acidity and the hemoglobin content of the blood, but they also noted the occurrence of severe anemia and a high value for hydrochloric acid. A more definite answer to this question was given by Davidson and Fullerton, who stated that hydrochloric acid can be secreted in the stomachs of persons suffering from extremely severe anemia of many types, clearly proving that anemia per se cannot be the primary cause of the achlorhydria.

A diminished secretion of hydrochloric acid is a common finding in chronic microcytic hypochromic anemia. Hartfall and Witts examined

137 patients and found extreme hypochlorhydria in 80 per cent and achylia that did not respond to histamine in 50 per cent. An incidence of achlorhydria in 64.9 per cent of cases of severe anemia of this type was reported by Davidson and Fullerton. The particulars of their findings are as follows:

	Per Cent
Histamine-fast achlorhydria	45.6
Achlorhydria after gruel; free hydrochloric acid after histamine	12.5
Achlorhydria after gruel; histamine not injected	6.8
Hypochlorhydria	14.6
Normal acidity	17.5
Hyperchlorhydria	3.0

The findings tabulated here concur with the reports of other investigations in disclosing that many reported cases of achlorhydria were not genuine instances of achlorhydria, as secretion of hydrochloric acid could be induced with histamine. Dhayagude and Khadilkar (1939) studied 75 cases of achlorhydria in association with hypochromic anemia and found that in half of this number though achlorhydria was present by the fractional test method, there was secretion of hydrochloric acid after histamine stimulation. Furthermore, there are records of other cases of histamine-fast achlorhydria in which repetition of the histamine injection yielded free hydrochloric acid. It is therefore not safe to base any prognostic predictions on the absence of hydrochloric acid after a single histamine test.

This type of anemia is particularly frequent among women of reproductive age and among infants. The immediate cause of such anemia is supposed to be iron deficiency, but actually the cause is not known. There have been reported several instances in which after treatment with massive doses of iron free hydrochloric acid was secreted in the gastric juice when the anemia was cured, although no hydrochloric acid had been found on one or repeated previous tests with histamine. It is thus possible, Hallen said, that the achylia in such cases is not the cause but the symptom of iron deficiency. In an article on chronic nutritional hypochromic anemia, covering 106 references, Davidson and Fullerton gave full consideration to the following hypotheses: 1. The achlorhydria is the result of the anemia. 2. It is neurogenic. 3. It is a constitutional abnormality. 4. It is the result of gastritis. As presented by them, the evidence is in favor of the hypothesis that the gastric defects and the nutritional changes are primarily conditioned by the same cause, namely, long-continued dietary deficiency. Adding to this the information presented in the preceding sections of this review, one may consider it as certain that functional and organic defects in the stomach are not the

primary cause of chronic microcytic hypochromic anemia. The frequency of anemia in women of child-bearing age necessitates reference to the gastric disturbance occurring in the course of gestation. Krahmer-Petersen (quoted by Ihre) found an 85 per cent decrease in the secretion of hydrochloric acid immediately before delivery, and within a few weeks he could demonstrate normal secretion of hydrochloric acid in almost all cases. Strauss and Castle studied gastric secretion during pregnancy and the puerperium in 24 women and found that 75 per cent of them did not secrete normal amounts of free hydrochloric acid and pepsin during more than half of the period of pregnancy. From these and other studies they concluded that the hypochromic anemia of pregnancy is due either directly to dietary deficiency or to deficiency conditioned by gastric anacidity, hypoacidity or associated gastrointestinal defects in the presence of fetal demands for blood-building material. Directly concerned with this subject are the studies on the anemia of pregnancy in gastrectomized and normal dogs reported by Bussabarger, Cuthbert and Ivy. Examining the blood pictures of 5 gastrectomized dogs through a total of 15 pregnancies, they found that marked to severe anemia developed in 11 pregnancies. The blood pictures of 12 normal dogs observed through 12 pregnancies showed marked anemia of pregnancy in only 1 case.

Quoted in various articles, Nothnagel (1879) and Fenwick (1880) have been credited with being the first to describe atrophy of the gastric mucosa in Addison's, or pernicious, anemia. However, according to Wilkinson, priority in such observation belongs to Flint (American), who, in 1860, pointed out the connection between the changes in the gastric mucosa and pernicious anemia in the following sentences: "Nor is it difficult to see how fatal anemia must follow an amount of degenerative disease reducing the amount of gastric juice so far that the assimilation of food is rendered wholly inadequate to the want of the body." It is generally recognized that the classic triad of pernicious anemia is (1) hyperchromic anemia, (2) degenerative changes in the spinal cord and (3) defective gastric secretion. Achylia is practically a constant finding, and it is understandable why so many authors take the stomach disease to be the primary cause of anemia. In the opinion of Hurst, the achlorhydria is an essential predisposing cause in about 99 per cent of cases of Addison's anemia.

In 1929 Castle described experiments which indicated that beef muscle recovered from a normal stomach after digestion for one hour had an effect similar to liver when administered to patients with pernicious anemia. His findings and those by Sturgis and Isaacs (1929) demonstrated that the stomach contains an antianemic (intrinsic) factor and

that it is the absence or diminution of this factor and not the achlorhydria that is important in the genesis of pernicious anemia. Wilkinson, Klein and Ashford incubated with beef steak stomachs of patients with a normal blood picture and stomachs of patients with pernicious anemia and noted that in the former case this was effective but that in the latter it was not. They concluded that the "enzyme" is absent from the stomach in pernicious anemia, or present in too small amounts to be detected by this method. These remarkable investigations still leave for consideration the importance of the stomach in the genesis of this disease.

Before discussing the matter of the relationship between the stomach and the intrinsic factor, attention will be given to other aspects of the functional changes observed in pernicious anemia. It is universally agreed that in this type of anemia both hydrochloric acid and pepsin are deficient. As reported by Wilkinson, who based his conclusions on fractional analyses of the stomach contents in 200 cases of pernicious anemia, the salient points in the secretory defects are as follows: The gastric contents are always of small volume, the average being 15.3 cc. Mucus is present, as a rule, in the fasting contents. In normal persons mucus is present in only very small amounts. The figures for the total acidity are low, the titer being approximately 5 to 10 units. The total chloride secretion follows the normal curve, though it shows general but not striking diminution. Following an injection of histamine, mucus is reduced to negligible quantities, emptying time is not influenced, the values for free and total acidity are not altered, chlorides may be increased, unchanged or diminished; peptic activities show no alterations from those obtained in the control analyses without histamine. While, as applied to normal animals, histamine may be the best test for determining the ability of gastric glands to secrete hydrochloric acid, dependence on it in testing the pathologically altered human stomach is not entirely safe. There are on record (Ihre) cases in which the stomach, while remaining refractory to histamine, has shown free hydrochloric acid as high as p_H 2.10 after vagal stimulation through insulin hypoglycemia. It may be, then, that if patients with stomachs refractory to histamine were tested with insulin, to the reported several dozen cases of pernicious anemia with free hydrochloric acid in the gastric contents more could be added. It is justifiable, therefore, to maintain that in a few cases of pernicious anemia the secretion of acid in the stomach is still satisfactory or can be restored under treatment, and the diagnosis pernicious anemia, as Alsted said, is not to be refuted perfunctorily because the patient shows free hydrochloric acid in the stomach contents. While free acid may occur in the gastric content of the patient with the typical hematologic picture of pernicious anemia, free acid is never found in the gastric content of the patient with undoubted posterolateral

sclerosis of the spinal cord. Oliver and Wilkinson reported that achylia gastrica occurred in all of their 39 patients with subacute combined degeneration of the spinal cord without anemia. It is interesting to recall at this time that achlorhydria is much more frequent in persons with alcoholism plus polyneuritis than in those with uncomplicated alcoholism.

Since the underlying cause of the secretory defect is due to degenerative changes in the nerves and the secretory elements, it is natural to expect that the production of pepsin must be damaged in anemia as much as the production of hydrochloric acid. Ihre reported 6 cases of pernicious anemia in which the application of the histamine and insulin tests yielded neither hydrochloric acid nor pepsin. Though in pernicious anemia peptic activities are generally considered as negligible, it is well to remember that, owing to the sensitivity of pepsin to alkaline mediums, the determination of peptic activities requires a great deal of care. Peptic activity when kept at p_H 6.5 for fifteen minutes is reduced 32 per cent and at p_H 7.4 is completely lost. With alkalinity greater than p_H 6.82, it is impossible to demonstrate peptic activity. Another practical suggestion related to this point was given by Faber, who stated that constant and complete cessation of all secretion was never observed by him when the test for pepsin was carried out on unfiltered fluid. Thus a failure to find pepsin in filtered and anacid gastric contents need not to be considered as absolute evidence that the pepsin-producing cells have been completely destroyed.

Since discovery of the intrinsic factor, in 1928-1929, a great deal of work has been done on the relationship between anemia, on one hand, and defects or disease of the stomach, on the other. As defined in the literature, the intrinsic factor is supposed to be a complex thermolabile substance, which, to judge from many facts, is a proteolytic enzyme and is not identifiable as hydrochloric acid, pepsin, pepsinogen, rennin or lipase. The factor is readily destroyed by heat, e. g., by boiling for five minutes and by heating to 70-80 C. for one-half hour. In their review of recent literature on the blood, Sturgis, Isaacs, Goldhamer and Bethel stated that it is generally accepted that the substance necessary to maturation of the red blood cells is produced by interaction of the extrinsic and intrinsic factors, with the essential part of this interaction occurring, according to Castle, in the small intestine. The resultant product is absorbed from the intestines, stored in the liver and released to the body tissues for utilization as needed. Earlier investigations showed that preparations from the pyloric gland regions were highly active, while those from the fundus gland region were inactive, and therefore it was first believed that the intrinsic factor was furnished by the pyloric part of the stomach, which was named as a "pyloric gland

organ" (Meulengracht). The idea of the pyloric gland region as a functional entity concerned with the production of the intrinsic factor has failed to withstand objective analysis. Surgical records dealing with gastrectomies did not disclose any frequency of pernicious anemia following gastrectomies. In most instances, simple microcytic hypochromic anemia was reported. Animal experimentation gave equally negative results. The most extensive studies along this line were made by Petri and associates. They reported that in dogs removal of the fundic region leads to slight anemia, of slow evolution, which becomes stationary or disappears completely in two to three months. Removal of the pylorus and of the entire duodenum produces progressive grave hypochromic anemia. Removal of the pylorus and of the region of the duodenum containing Brunner's glands provokes slowly hypochromic anemia with a discrete tendency toward polycythemia or hyperchromic anemia without megalocytes. As observed in young swine, gastrectomy has led to the development of hypochromic microcytic anemia. Bachrach and Fogelson removed gastric tissue well beyond the limits of the pyloric glands, with none of the dogs operated on showing any evidence of pernicious anemia. Though their 7 dogs were observed for a period of over two years after radical subtotal gastrectomy, complete duodenectomy and partial jejunectomy, no evidence of pernicious anemia was shown by any of the animals operated on. They concluded, therefore, that the "pyloric gland organ" in the dog is not essential for the maintenance of a normal blood picture. Particularly strong evidence against the gastric theory is brought up by the finding that extracts made of other parts of the gastrointestinal tract contain a similar antianemic factor. Uotila found that the small intestine has more antianemic principle than the stomach and that the strength of the antianemic activity varied in the ileum, duodenum and jejunum in decreasing order. Erös and Schemensky reported that extracts from the colon also possess considerable antianemic property.

Reports covering morphologic changes observed in association with pernicious anemia offer a great deal of valuable information for the understanding of functional gastric defects occurring with this disease. Considerable attention has been given in recent years to examination of the mucosa with the aid of the gastroscope, and it is worth while to mention some general information obtained with this method. Jones, Benedict and Hampton recorded the results of gastroscopic, surgical and roentgenologic examinations in 5 typical cases of pernicious anemia which were observed for a long period. They presented evidence which in their opinion indicates that atrophy of the mucosa occurs particularly during a relapse and is not an invariable accompaniment of the disease. They observed hypertrophic gastritis during a relapse and during the

early stages of a remission, but after specific treatment of the anemia both the atrophy and the hypertrophy of the gastric mucosa tended to disappear. Schindler and Serby made gastroscopic observations in 23 cases of pernicious anemia and reported more or less extensive atrophic changes found either throughout the stomach or in the body alone; the antrum sometimes may be free from changes. Of particular interest is their case 22, in which after three months there was complete regeneration of the mucosa. There have been numerous investigators of the morphologic aspects of the stomach in association with pernicious anemia, and whether their histologic technic was good or bad, they all have been unanimous in the conclusion that the most common finding in this disease is atrophy of the functional mucosa. In 1900 Faber and Bloch published the first of the papers in which they described their findings in material fixed soon after death. They examined stomachs from 8 patients who died from pernicious anemia and found diffuse gastritis in every one. Wallgren (quoted by Magnus and Ungley) examined the gastrointestinal tract in 16 cases of pernicious anemia and 2 cases of aplastic anemia. Diffuse atrophy of the gastric mucosa was present in all the cases of pernicious anemia, whereas the stomach was normal in the 2 cases of aplastic anemia. Brown analyzed the autopsy records in 42 cases of pernicious anemia and stated that the picture of chronic gastritis was present in all but 1 case and that oxyntic cells were absent in all but 5 cases. Studies by Magnus and Ungley on properly fixed material and backed by their authority in judgment deserve particular consideration. Their studies cover 7 cases of pernicious anemia, in 1 of which there was also subacute combined degeneration of the spinal cord. The gross changes were identical in all cases: the upper two thirds of the stomach, i. e., the mucosa in the region of the body of the stomach, showed atrophy so severe that this area was reduced to the thinness of parchment. At the junction of the body mucosa with the pyloric mucosa, however, there was a transition, usually abrupt, to the normal thickness of the stomach wall, and this was maintained throughout the whole of the pyloric antrum and duodenum, with the pyloric mucosal surface appearing normal. Microscopically, all that remained in the mucosa was the surface epithelium plus a few scattered glands lined by mucus-producing cells, while the specialized oxyntic and peptic cells had entirely disappeared. Yet, in spite of this, there was a complete absence of fibrosis, cellular infiltration, obliterative endarteritis or other evidence of past inflammation, and there was a corresponding absence of fibrosis in the submucosa and subserosa. The muscle coat showed atrophy, but here also there was absence of fibrosis. Several of the stomachs exhibited large areas of

intestinal epithelium in the atrophic body mucosa but not in the normal pyloric mucosa; this is to be regarded as an example of metaplasia. Pyloric and duodenal mucosa showed no abnormality, and in connection with this finding they said: "There is, therefore, a morbid anatomical basis for the achylia gastrica present in pernicious anemia, but not for the absence of the intrinsic factor if we assume that it is produced in the pyloric region." The lesion observed affected all the coats of the stomach wall and from its histologic appearance is almost certainly not the end result of an inflammatory process but is to be regarded as an atrophic process, the cause of which is not yet known, but which may be the end result of some endocrine or nutritional deficiency or may even be congenital in origin. While the progressive disappearance of specialized cells of the mucosa is the most important and most common pathologic change found, there are numerous reports of hyperplastic types of lesions being found in association with this disease. The gastric polyps (Brown; Ungley; Haring), found mostly in the pyloric region, are interpreted as the result of gastritis, and some have suggested polyposis as a causative factor of anemia. The objective evidence in support of this supposition is meager, as is that in support of the opinion expressed by some observers that gastric carcinoma is of frequent occurrence in patients with pernicious anemia. Conner, quoted by Berger, found in the files of the Mayo Clinic between 1925 and 1932 only 11 cases of gastric carcinoma in association with pernicious anemia. According to Wilkinson, the occasional association of pernicious anemia with carcinoma of the stomach is probably fortuitous.

From the earliest days it has been recognized that gastric disturbances are frequently present in the anemia that is caused by Diphyllobothrium latum, which clinically, hematologically and pathologically is indistinguishable from cryptogenic pernicious anemia. It is therefore of interest to review the essentials of the functional and organic disease observed in this type of anemia. Probably the first man to link together pernicious anemia and helminthiasis was Albrecht, pathologist in St. Petersburg, Russia, who, even before the time of the adoption of anthelmintic therapy, used to mark in protocols of necropsies under diagnosis in such cases "Helminthiasis–Anemia Perniciosa." In reference to the functional disturbances, the reports published show that while there is an insufficiency of gastric secretion of hydrochloric acid in patients with anemia, in carriers without anemia gastric acidity is normal. Most of the information given is taken from an excellent article by Birkeland, which is entitled "Bothriocephalus Anemia" and covers 485 references. Birkeland carried out estimations of acidity in 87 cases, with achlorhydria being found in 83.9 per cent and free hydrochloric acid in 16.1 per cent.

He has given the following tabulation of the age distribution of the anacidity in Dibothriocephalus anemia:

Age	Percentage
0-20	53.8
21-30	73.3
31-40	90
41-50	95
51-60	91.6
61-70	100

As given by various investigators, figures show that the occurrence of anacidity in this type of anemia fluctuates much more and is less constant than in pernicious anemia. Furthermore, the patients with achlorhydria quite often show a return of acidity after the worm is expelled and the anemia cured. In the opinion of the majority, the postmortem changes in Dibothriocephalus anemia are identical with those in pernicious anemia. In both types of anemia, as already disclosed, there is a great deal of similarity, yet there are differences which remain unexplained. After systematic anthelmintic measures were introduced (1885), the mortality was reduced from 100 to 12 per cent. It is true that some of the patients die from the anemia despite removal of the worm, and some of them after recovery succumb to pernicious anemia later in the absence of any worm. Yet it is equally true that in many instances the expulsion of the worm is followed by rapid clinical and hematologic improvement, accompanied by typical reticulocytosis, the character of which is similar to that observed in response to liver or stomach preparations. Since no conclusive proof has been offered to support the claim that the worm produces or contains a specific hematoxin, there must be other factors which cause the anemia and which are responsible for the improvement which follows expulsion of the worm. An interesting approach to the solution of this problem was made by von Bonsdorff, who investigated the comparative action of intestinal worms on liver extract and on the proteolytic activity of normal human gastric juice. The results obtained by him show that a liver preparation which was exposed in vitro to fresh Diphyllobothrium latum, Taenia saginata and Ascaris lumbricoides, to boiled Diphyllobothrium latum and to alcohol extracts of Diphyllobothrium latum and of Bacillus coli did not lose demonstrably the antianemic effect through any of these treatments. Contrary to this, aqueous extracts of fresh and dried Diphyllobothrium latum and Taenia saginata and of fresh Ascaris lumbricoides exercise a marked inhibiting influence on the proteolytic activity of the normal gastric juice at a reaction in the vicinity of the

neutral point. The same substances do not inhibit the action of trypsin, papain and pepsin and of gastric juice at strong acid reaction. Assuming that the gastric enzyme, active at neutral reactions, can be proved identical with Castle's "intrinsic factor," it is clear that the results of von Bonsdorff's work can be of importance for the explanation of the genesis of pernicious worm anemia.

Because of its close relation to the subject discussed, a brief reference will be made to the gastric changes observed in association with sprue, a disease which probably is caused by a dietary deficiency (Castle and associates). Digestive disturbances are by far the most common vagaries seen in patients with tropical sprue. Ashford found achylia in 39 per cent, the average p_H value in 26 cases being 2.64 and the total acidity in 19 cases representing all grades of the disease being at first reading 26.7. Scheer (quoted by Alsted) found achylia in only 4 of 33 patients with tropical sprue, and all 4 had normal acidity values on recovery. In the opinion of various investigators, the gastric mucosa may show certain grossly visible changes. Using the gastroscopic method of examination, Rodriguez Olleros recently reported atrophic gastritis in one form or another, sometimes generalized, sometimes localized, in 21 of 28 cases. Neither functionally nor morphologically was the extent of damage nearly as great as that observed in pernicious anemia. While studying the elimination of neutral red by the gastric mucosa of patients with tropical sprue, Rodriguez Olleros observed that in spite of the atrophy of the mucosa the dye was eliminated. In contrast to this, in pernicious anemia elimination of neutral red does not take place. It is well to remember this useful test, which became available as a result of studies by Harvey and Bensley, who showed that neutral red is eliminated by the gastric mucosa. The elimination of dyes by the gastric mucosa has since been studied thoroughly by Dawson and Ivy, who on injecting intravenously 33 different dyes found that 13 were eliminated in the gastric juice. In their opinion, the parietal cells are the cells chiefly concerned in the elimination of dyes. Attempts to apply dye elimination as a diagnostic test for various gastric ailments were made by a number of investigators. If regurgitation from the intestine is ruled out by testing the gastric contents for bile, the Fricker test (1923), based on the occurrence of indigo carmine in the gastric contents of patients with ulcer and carcinoma, may be of value, since this dye is not eliminated by normal gastric mucosa.

GENERAL CONSIDERATIONS

The preceding reviews open an approach to the interpretation of functional and organic disease of the stomach. They show that the stomach is a unique organ in its sensitivity to the effects of various

factors and that there are reasons to believe that variations in secretory activity may occur in the stomach free of disease. On examining "healthy" people, said Keefer and Bloomfield, every degree may be encountered from the conventional normal values of acidity to the very low figures. The actual normal is difficult to define, and the question may be raised whether absence of free hydrochloric acid can, in any sense, be regarded as abnormal unless there are other evidences of gastric disease or deficiency. Even among the groups of "supernormal" people approximately 1 in 20 has deficient gastric secretion. As applied to "supernormal" people, the term "deficient gastric secretion" used by Keefer and Bloomfield is not fully justifiable. Taking into consideration both the functional life cycle of the parietal cells and the environmental conditions which can influence the function of these cells, variations in acidity are to be expected in every normal animal, and they do not need to be regarded as a manifestation of pathologic deficiency. A single gastric analysis, or even three or four made on successive days, may give a decidedly misleading idea of the usual ability of the particular stomach to secrete acid. This is well demonstrated by Alvarez and Vanzant in their studies on daily variations in the concentration of acid and pepsin.

There is general agreement regarding the relationship between hypoacidity and anacidity, on one hand, and age, on the other. The life span of the parietal cells appears to be rather short, and it is fairly uncommon to find normal production of acid after the age of 35. In about 40 per cent of all persons above the age of 50 the acid secretion is either diminished or has ceased. Emphasizing that examinations were made of healthy old people, Dedichen found gastric anacidity in 66 per cent of 90 persons between the ages of 67 and 92. There are some conflicting reports as to the extent of the variations in acidity, especially after the histamine test; but, on the whole, age and the progressive senile atrophy of the functional mucosa which develops with advancement in age are shown to be related definitely to general gastric hypofunction and anacidity. The sum of results presented in the preceding reviews shows that pathologic deviations in acid and pepsin concentration are invariably in the direction of a decrease. There is no disease capable of inducing true gastric hyperacidity, and essential hypersecretion (Reichmann's disease) probably does not exist (Carlson). The factors definitely known to induce hypersecretion and hyperacidity are: delayed gastric evacuation and a failure on the part of the acidity-reducing factors.

Accessible to objective estimations of their functional efficiency, the specialized cells of the gastric mucosa offer an opportunity for studies which cannot be applied to many unapproachable organs of the body. These studies, based on combined physiologic and cytologic evidences,

demonstrate that cessation of the production of hydrochloric acid can occur in the absence of structural changes in the parietal cells. The functional achylia can be provoked by such unrelated factors as thyrotoxin, the roentgen ray, alcohol, diphtheria, toxin, barbiturates, vagotomy and a rise in temperature caused by infection or by injection of foreign proteins. Temporary inhibition of the production of hydrochloric acid is observed under the influence of extracts (enterogastrone or chalone) from normal urine and under that of extracts from the gastric juice of patients with pernicious anemia and carcinoma. Supplemented with the explanation of the underlying mechanism, the inhibitory effect of thyocyanate is of particular importance. In this case the degree of inhibition of acid secretion can be predicted from a knowledge of the nature of the inhibition of carbonic anhydrase in the parietal cells.

In some instances injury is followed by structural changes in chief and parietal cells. It appears that in their response to injury these two types of cells react somewhat differently. Besides evidence of actual destruction, the chief cells show a specific type of response to injury. This response is manifested by gradual transformation, or cytomorphosis, which follows a course of reversed ontogenetic order. The chief cell dedifferentiates first into a mucous neck type cell, then into a mucoid cell and possibly even into a primitive nonmucoid cell. As reviewed in previous sections, such cytomorphosis of dedifferentiation can be conditioned by a number of factors. Cytologic evidence indicates that this process is not irreversible, and whenever enviromental conditions permit it, the cell can redifferentiate and reassume the morphologic and functional characters of a chief cell. Under pathologic conditions this process of redifferentiation varies. It may either fail entirely, or it may be incomplete, or, as happens quite often, the redifferentiation may take a course in the direction of the intestinal pattern.

The response of parietal cells to injury is not traced with the same degree of certainty. It appears that the functionally mature parietal cell is the cell which is damaged first. This damage is manifested by hydropic and vacuolar degeneration, seen chiefly in the middle zone of the functional mucosa. The immature and rejuvenating parietal cells are least sensitive to injury, and they serve as a source of parietal cells which replace the damaged elements. Both the instability of the specialized cells of the gastric mucosa and the types of their response to injury make understandable the difficulties in interpreting functional deviations observed in pathologic stomachs. It is obvious that physiologic standards of evaluating acid production and acidity-reducing factors cannot be applied to the diseased mucosa replaced with the metaplastic type of lining and showing only a few parietal cells left intact.

In addition to metaplasia, the cellular infiltration, or so-called gastritis, cannot be disregarded in interpreting functional and organic disease of the stomach. The reported findings show that lymphocytic infiltration of the mucosa and submucosa is observed in the course of various experiments and in association with all kinds of gastric disease. In rats such infiltration is a common manifestation of nutritional deficiencies, and it is found along the entire gastrointestinal tract. The question arises whether in this case it is allowable to speak of such infiltration as gastritis. If this is to be designated as gastritis in the sense of inflammation, what should one call the extensive lymphocytic infiltration of gastric mucosa found constantly in certain animals during hibernation? Faber (1927) has asserted since 1904 that achylia is a disease depending on exogenous factors and developing from gastritis and that anacidity begins at such an early stage that atrophy is out of the question, the inflammatory changes being the only changes found. He expressed the belief, also, that it is in gastritis one must seek the origin of the chronic ulcer and that the frequent ulcerative pyloric gastritis is the cause of the juxtapyloric ulcer. The inflammatory basis of both ulcer and carcinoma is supported by Konjetzny. The most frequently mentioned theory of the gastric ulcer is that of Rokitansky and Virchow, which postulates an organic or functional closure of the vessels with resultant hemorrhagic or anemic necrosis and digestion of the necrotic tissue by the gastric juice. According to Aschoff, five theories must be considered in the etiology of the erosion: (1) the inflammatory theory, (2) the theory of displacement of mucosa, (3) the spastic theory, (4) the circulatory theory and (5) the mechanical theory. The remark just made concerning a possible relationship between gastritis, peptic ulcer and carcinoma justifies saying that the matter of such relationship is far from being established. While the inflammatory, hyperplastic and ulcerative lesions can be easily produced in animals by a variety of factors, carcinoma of the stomach is the rarest condition observed in animals. According to a review of experimental gastric carcinoma by Klein and Palmer (1940), there is no well established case of adenocarcinoma of the stomach produced experimentally. Are the methods of experimental approach unsuitable, or is this failure due to the insurmountable resistance on the part of the animal? These questions are left to be answered by future research.

With the desire to avoid controversies centered around certain and hitherto unsolved clinical problems, this review is made primarily with the purpose of presenting the basic knowledge, which is assembled in a way easily accessible to those interested in the clinical and research aspects of gastric pathology. Each section of the reviews presented is in itself a summmary requiring no additional comments.

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Notes and News

University News, Promotions, Resignations and Appointments.—H. E. Meleney, associate professor of preventive medicine and public health in Vanderbilt University, has been appointed Hermann M. Biggs professor of preventive medicine in New York University.

S. Harvey Colvin Jr., formerly of the Piedmont Hospital and the Grady Hospital, Atlanta, Ga., who had his training at Touro Infirmary, New Orleans, and at the Mayo Clinic, has been appointed pathologist at Touro Infirmary, succeeding John A. Lanford.

Robert D. Defries, associate director of Connaught Laboratories, University of Toronto, has been appointed director to succeed the late John G. Fitz Gerald.

F. A. McJunkin, professor of pathology in the Loyola University School of Medicine since 1927, has retired, having reached the age limit.

N. Paul Hudson, professor of bacteriology at Ohio State University, has joined the Rockefeller Foundation Health Commission in France, on a year's leave of absence.

Deaths.—Oluf Thomsen, professor in the University of Copenhagen, whose contributions in the fields of infection, immunity and blood are well known, died in June 1940, at the age of 62. He was one of the founders and editor of *Acta pathologica et microbiologica Scandinavica.*

Ernest A. Pribram, professor of clinical microscopy in Loyola University School of Medicine and pathologist to St. Elizabeth Hospital, Chicago, and formerly assistant to the director of the State Serum Institute in Vienna and associate professor of pathology in the University of Vienna, was killed in an automobile accident on Sept. 14, 1940, at the age of 61 years. Dr. Pribram was an active investigator in pathology and bacteriology. His work dealt mainly with biochemical problems and with the classification of fungi.

Medals and Awards.—Herbert C. Clark, director of the Gorgas Memorial Laboratory of Tropical and Preventive Medicine, Panama, R. P., received the Walter Reed Medal of the American Society of Tropical Medicine at the annual meeting in Louisville, Ky., in November, in recognition of his years of work in the eradication of malaria and other tropical diseases.

Béla Schick, pediatrician-in-chief at the Mount Sinai Hospital, New York, received a gold medal for "distinguished and outstanding contributions in the field of allergy" at the third annual forum on progress in the study of allergy, held in Indianapolis, Jan. 11 and 12, 1941.

The Gold Medal of the American Institute of New York for 1941 has been awarded to Wendell M. Stanley, of the Rockefeller Institute for Medical Research at Princeton, N. J., "for crystallizing the virus of tobacco mosaic . . ."

Society News.—At the annual meeting of the Illinois Society of Pathologists Dec. 7, 1940, Oscar T. Schultz was elected president and I. Davidsohn secretary-treasurer.

The annual meeting of the American Association of Pathologists and Bacteriologists will be held at New York University, 477 First Avenue, New York, April 10 and 11. One-half day will be devoted to the subject of shock. Contributions to the program should be in the hands of the secretary, Howard T. Karsner, 2085 Adelbert Road, Cleveland, not later than March 8, 1941. The headquarters will be the Hotel Biltmore.

Oswald T. Avery, member of the Rockefeller Institute for Medical Research, was elected president of the Society of American Bacteriologists at the opening of its annual convention in Cincinnati, on December 27.

Obituaries

HANS ZINSSER, M.D.

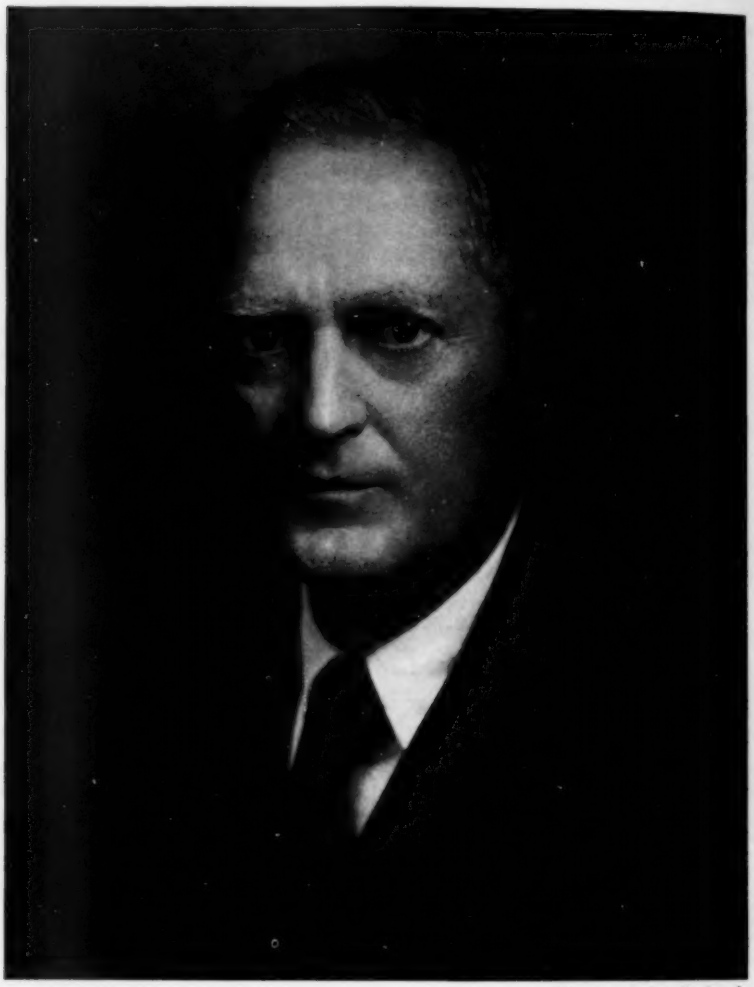
1878-1940

Since Dr. Zinsser's death on Sept. 4, 1940, eloquent tributes have been paid to his memory by his lifetime friends, his associates of the laboratory, his pupils and the medical students of his last classes. While all have been moved by remembrance of the paradoxic harmony of the varied activities of his life, each writer of a biographic note has emphasized one or another of his qualities as philosopher, scientist, organizer, executive, man of letters, musician, poet, sportsman, lover of the out-of-doors, and endeared companion. These are the natural responses of different men who, while sharing the same affection and regard for him, found in the riches of his personality some treasure of special value to the individual. As President Keppel wrote: "He was as many-sided as Benjamin Franklin, but despite the wide sweep of his interests his life was not cut up into separate compartments, but on the contrary it was singularly unified." After noting that some "see him as a great teacher, others as a distinguished investigator, many as a perspicuous and sensitive commentator on science, letters, education, and on his own and others' lives, . . . a poet of great emotional intensity," Dr. Enders writes of him mostly "as head of the laboratory; to depict . . . some of those qualities of mind and heart which enabled him to fashion that office into a remarkable instrument whereby not merely bacteriology and immunology were advanced, but the lives of those who came to him were broadened and enriched."

Throughout his life Hans Zinsser showed superb courage, whether in the field or in the laboratory, whether in attack on powerful popular movements in medical education or in defense of his ideals. The finest expression of his steadfastness and bravery was given in the way he conducted his life after June 1938, when he found that he had lymphatic leukemia. Although he knew for two years that he was suffering from an incurable disease, he never faltered in his gallant course. On the contrary, he worked as hard as he could. He pushed forward his investigations. He taught with characteristic enthusiasm. He completed and published "As I Remember Him," his moving, delightful, philosophic semiautobiography. The manner in which he met his death was in the tradition of his code, and it added to the nobility of human life.

When he died, shortly before his sixty-second birthday, Dr. Zinsser was in the full tide of scientific and literary productivity. As Dr. Mueller

has written, after speaking of the versatility of his genius and of his contributions, "Seldom is it possible in a brief span of years to penetrate such diverse ways. Rarely can death inflict a greater loss."



Photograph by Bachrach.

HANS ZINSSER, M.D.
1878-1940

GENERAL OUTLINE OF DR. ZINSSER'S LIFE

Hans Zinsser, the youngest of four sons, was born in New York on Nov. 17, 1878. His father, August Zinsser, a chemist, came to America from the Rhineland because he regarded this country as "the promised land where there was still room for all free spirits whose wings were

clipped at home by bigotry and political oppression." His mother, Marie Theresia (Schmidt), was the daughter of a "political dissenter in the Black Forest." His parents "became politically Americans with a speed and thoroughness surprising to all who did not understand their fervid admiration of our institutions." As a member of this family of cultivated people, Hans Zinsser grew up in an environment of liberalism, broad interests, vital human associations, appreciation of values, and the traditions of unremitting honest work.

After the completion of his primary education under tutors and at private schools in Germany and in New York, Hans Zinsser entered Columbia University in 1895. Of his college education he wrote that it "consisted of Woodbury, Wilson and Dean—thus affirming the truism that one is trained by men and not by curricula." While he was in college the Spanish American War broke out, and in spite of his youth he enlisted in Squadron A of the New York Cavalry. He never got to the front, but he learned at first hand of the "unbelievably miserable sanitary supervision of the camps." Forever after he had a keen interest in military sanitation, and from this developed his concern with world-wide public health. He received the degree of Bachelor of Arts from Columbia in 1899 and the degree of Master of Arts in 1903. In the same year, 1903, he was graduated from the College of Physicians and Surgeons of Columbia University, with the degree of Doctor of Medicine.

In June 1905, two years after his graduation from medical school, he married Ruby Handforth Kunz, of New York. Dr. and Mrs. Zinsser had two children, a son, Hans Jr., and a daughter, Gretel, now Mrs. Vernon Munroe Jr.

From 1903 to 1905 Dr. Zinsser was a member of the House Staff of the Roosevelt Hospital and bacteriologist of that hospital during the year 1905-1906. In 1905 he received his first academic appointment as assistant bacteriologist at Columbia University. Two years later, in 1908, he was promoted to the position of instructor in bacteriology. From this position in 1910, at the age of 32, he went to California to be associate professor of bacteriology and immunology at Leland Stanford University. During the previous three years, 1907 to 1910, he was assistant pathologist at St. Luke's Hospital, New York.

In 1913 Dr. Zinsser returned to Columbia as professor of bacteriology and immunology, a position which he occupied for the next ten years. During the same period he served as bacteriologist of the Presbyterian Hospital in New York.

He became professor of bacteriology and immunology at Harvard University Medical School in 1923, and in 1925, at Harvard, he was given the distinguished Charles Wilder professorship. During the thirteen years of his residence in Boston he had numerous appointments and associations through which he kept in touch with the clinical side

of infectious diseases. Among these were his appointments as chief bacteriologist of the Children's Hospital, the Infants' Hospital and the Peter Bent Brigham Hospital.

Several other dates are significant in his life and in his scientific career. In 1915, feeling strongly that he should "get into the war in some capacity," he joined the Red Cross Typhus Commission for Serbia and went abroad. His experiences in the Serbian epidemic, "as terrifying and tragic an episode as has occurred since the Middle Ages," brought him into close contact with "mass misery" for the first time. This expedition profoundly influenced his thinking for the rest of his life. It aroused his interest in problems of typhus fever, on which he worked intensively during his last ten years. Another result was his return to typhus-infested regions eight years later, when he went to Russia in 1923 as a sanitary commissioner for the Health Section of the League of Nations. In these two expeditions he gathered the material he used for the "biography" of typhus, his book called *"Rats, Lice and History,"* published in 1935. The interest in typhus fever stimulated in 1915 was in large part the cause of his going to France in 1935 as exchange professor at the University of Paris, of his visit to his dear friend Charles Nicolle in Algiers and of his journey to China in 1938 for a period of teaching as visiting professor at the Peiping Union Medical College.

The entry of the United States into the World War in 1917 presented an opportunity for service which Dr. Zinsser accepted immediately and developed to the full extent of his knowledge, skill and energy. He was commissioned major in the medical corps of the United States Army in 1917 and promoted to colonel in 1918. He served in France with the American Expeditionary Forces as sanitary inspector of the First Corps and later of the Second Field Army. During a portion of his two years in the A. E. F., from 1917 to 1919, he was assistant director of the Division of Laboratories and Infectious Diseases. The thoroughness of his inspections under conditions of danger and discomfort and his lashing condemnations of breaches of sanitary regulations are vividly remembered by all who saw him in action. With his usual comprehension, he grasped the breadth of the problems of military sanitation, understanding their relationship to the hygiene of the individual and to general public health. The orders issued on his recommendation in the Second Army became a treatise, published in 1919, entitled "The Sanitation of a Field Army." His observation of epidemic influenza and of cases of encephalitis which occurred during the war formed points of departure for investigations in subsequent years. His interest in military sanitation, aroused first by events of the Spanish American War in 1898, developed later through his practical experience in the World War

from 1917 to 1919, was unabated at the time of his death in 1940, when this country was beginning to prepare again for its own defense. His last paper, published just before he died, was "On the Medical Control of Mobilization." In discussing plans for mobilization under the Compulsory Selective Service Act he brought his knowledge of epidemiology to bear on problems which were about to be presented by the herding of young men into training camps. He advanced the idea that small groups of soldiers should be formed first in regions near their homes and that, after a period of seasoning, these groups should be brought gradually into larger and larger assemblages. He believed that in this way disastrous outbreaks of infectious diseases could be prevented among both the recruits and the older troops.

For his achievements in the World War, Dr. Zinsser was awarded the United States Army's Distinguished Service Medal and the Order of St. Sava by Serbia. Years later, in 1935, in appreciation of his distinguished services in the war and in recognition of his many accomplishments, he was decorated by the French government with the Legion d'Honneur, in the rank of chevalier.

Dr. Zinsser's distinction as a scientist and as a man of great attainments was recognized by the award of the honarary degree of Doctor of Science by Columbia University in 1929, Northwestern University in 1931, Lehigh University in 1937, Yale University in 1939 and Harvard University in 1939.

Although Dr. Zinsser was a strong individualist and may be said to have fought to preserve his time for scientific research, literary writing, personal associations, and for the kind of strenuous recreation which he enjoyed, he gave a large amount of time and effort to the service of many organizations. It is astonishing to see the length of even a partial list of the committees, boards and organizations to which he belonged. Among his numerous serviceable offices was membership on the Board of Trustees of the Massachusetts General Hospital. He held positions on the editorial boards of five scientific and medical journals. He belonged to upward of a dozen fraternities, honorary societies and clubs. He was a member of some thirty-five or more scientific societies in this country and abroad. Among these was the National Academy of Science. He was president of the New York Pathological Society in 1915, of the American Association of Immunologists in 1919 and of the Society of American Bacteriologists in 1926.

DR. ZINSSER'S SCIENTIFIC WORK

In this section an attempt will be made to summarize Dr. Zinsser's scientific work and to venture an appraisal to the extent at least of pointing out the setting, methods and significance of his most important contributions. At this time, so soon after Dr. Zinsser's death, it is

obviously impossible for any reviewer to write with finality. The full meaning of a scientist's career cannot be appreciated until after the passage of years, during which the results of his work are either confirmed or revised by the experiments of others and during which values are apt to be shifted. For personal reasons I cannot write objectively about the man who was my friend and master for so many years. Furthermore, I must give notice of lack of knowledge. Bearing these things in mind, the reader will know what weight to give to the opinions here expressed.

During thirty-seven years, from 1903 to 1940, Dr. Zinsser published about 180 scientific papers, according to a bibliography not yet completed. In addition, he published numerous reviews, biographic articles, papers on medical education and general papers on public health, many of which contain scientific observations. Since 1910 his "Textbook of Bacteriology," begun in collaboration with Philip Hanson Hiss Jr. and continued in association with me, has gone through eight editions and thirty-eight printings. His book "Infection and Resistance," published first in 1914, passed through three editions under that title, a fourth edition in 1931, under the title "Resistance to Infectious Diseases," and a fifth edition in 1939, with the title "Immunity, Principles and Application in Medicine and Public Health," written in collaboration with John F. Enders and LeRoy D. Fothergill. Furthermore, his semifictional volumes, "Rats, Lice and History," published in 1935, and "As I Remember Him," published in 1940, and the poems of R. S. in the *Atlantic Monthly* belong in part to his scientific writings. All of these, as well as his papers in scientific journals, are mentioned as sources, because scattered through them, unpublished elsewhere, are his own comments and indication of his attitude toward work in progress. The biographer who will deal fully with Dr. Zinsser's scientific work must of necessity analyze and collate this mass of material.

Dr. Zinsser's scientific work may be divided into periods in accordance with the main events of his life as outlined in the first section of this article. Looked at from this point of view it has a somewhat episodic character. Investigations appear to have had their origins in problems presented by particular cases or epidemics, by the events of an expedition or by current developments in some field of bacteriology or immunology. Some researches appear to have been intentionally put in motion to capture some strong point of practical advantage in the battle against infectious disease. Although, as he said of R. S., "his heart was in the laboratory," it is clear that his eyes were often fixed on the applications of new knowledge. To point out that he had such objectives is not by any means to imply that he was not deeply concerned far beyond the extent of his scientific training with the fundamental

biologic and chemical problems which underlie bacteriology and immunology. Intensely interested in basic principles, he went as far as he could to get at them in connection with whatever immediate specific problem was engaging his attention. Actually, from the point of view of objectives and methods of attack his work has a sustained coherence which is not apparent from the mere inspection of the titles of his papers.

Dr. Zinsser's first paper, in 1903, dealt with the effects of radium on bacteria. It was followed during the next five years with accounts of interesting cases of infection. His first series of reports of work done in a planned program of investigation were the papers published from 1908 to 1911, in collaboration with Philip Hanson Hiss Jr., on the preparation of extracts of leukocytes and on the effects and uses of these extracts in the treatment of infections in man. This work, first started by Dr. Hiss, followed logically on the discovery of Denys and Kaisin, and others, that leukocytic extracts have bactericidal properties. Hiss and Zinsser were able to show that the extracts did not favor phagocytosis and that their moderate bactericidal activity could not account for their effectiveness. Reviewing this work in 1940, Dr. Zinsser wrote that he was "inclined to believe that the beneficial effects are to be attributed to those obscure factors which account for the not infrequent successes of so-called nonspecific protein therapy, which consists in the injection of almost any bacterial or other protein."

As an indication of Dr. Zinsser's clinical interest in a field outside bacteriology, it should be mentioned here that in 1908 he and Dr. Karl M. Vogel published a translation from the German of a volume by Dr. Rudolph Schmidt entitled "Pain, Its Causation and Diagnostic Significance in Internal Diseases." The translators added a chapter of their own, summarizing the current researches of Head on referred pains. This volume is of especial interest to biographers, because it is not unlikely that the mysterious R. S., Dr. Zinsser's *nom de plume* of later years, may have materialized in a whimsical manner from some element of association with the title-page of this book.

At about the time when Dr. Zinsser went to Leland Stanford University, in 1910, he was becoming increasingly interested in the nature of antigens and antibodies, in the mechanisms of their reactions and in their effects on organisms from bacteria to man. Out of the investigations prompted by this interest came a long series of papers reporting important discoveries and several unifying theoretic conceptions. The researches led into fields of physical chemistry and biochemistry and into studies of different states of hypersensitivity, allergy and anaphylaxis. At Stanford University he was associated with Prof. Stewart W. Young, who collaborated with him in developing the colloidal chemistry of immune reactions. From these conceptions of colloidal and physical

chemistry and from experimental immunologic tests, it appears that Dr. Zinsser developed, in 1912, his "unitarian view" of the essential identity of the antibodies. The view that precipitins, agglutinins, sensitizers and other factors of like character are in general the same antibody, given a different name in accordance with its effect on different states of antigens, brought together previous observations of a number of workers. On the basis of his independent work, Dr. Zinsser's ideas agreed essentially with those of Dean, whose Croonian Lecture of 1912 was on this subject.

During this period he began to get interested in the sizes of particles and the role of surface phenomena in serologic reactions. For many years, thereafter, recurring to the theme of these early experiments, he used the methods and knowledge thus gained to solve new problems. He ventured even to write a mathematical paper on the physical chemistry of antigen-antibody reactions.

During the period from 1894 to 1906 the fundamental observations on hypersensitiveness had been made, the chief characteristics of anaphylactic reactions to foreign proteins were known, and other states of allergy to substances of diverse origin, including bacterial substances, were outlined. By 1910, the publications in this field were becoming numerous, and leading immunologists were advancing theories based on highly ingenious and intricate experiments. The field of the allergies, now known to be vast, was being opened by pioneers. Entering into investigations of anaphylaxis and bacterial hypersensitivity with all his zest and skill, Dr. Zinsser, within a few years after his return to Columbia in 1913, became one of the chief authorities on the subject. He did much to clarify ideas by his experiments confirming the view that anaphylaxis is mediated by a reaction between antigen and antibody. In the field of bacterial hypersensitiveness he made discoveries.

The tuberculin reaction was an instance of hypersensitivity which interested him particularly. He made a thorough investigation of the mechanism of this reaction and of similar examples of specific hypersensitiveness in bacterial infections. In addition to finding explanations of these states of allergy, he discovered the reactive nonprotein materials, the "residue antigens," a recognition of which forms a part of the revolutionary developments in immunology, which have come from studies of the "haptenes" predicted by Landsteiner.

During the period from 1916 to 1921 Dr. Zinsser in collaboration with Parker, Mueller and others was accumulating observations on the characteristics of what he called "residue antigens" from the tubercle bacillus and other bacteria. This material, the residue left when alkaline extracts of bacteria were boiled with acid, was antigenic in the sense that it reacted with antibodies and produced specific local effects and

shock when injected into hypersensitive animals. It did not, however, elicit the production of antibodies and hence was not a complete antigen. The studies with the residue antigen of tubercle bacilli confirmed the earlier suggestion of Landsteiner concernnig the possible nature of tuberculin. The independent observations of Zinsser and his associates took their place also beside the investigations of Dochez and Avery, published in 1917, on the occurrence of specific precipitable substances in the blood and urine of patients with pneumonia. Finally, they formed a link with the experiments reported in 1912 by Pick, to whom Dr. Zinsser, in a later discussion, gave the credit for being "actually the first to observe the bacterial haptophore substances which have since then revolutionized our conceptions of the bacterial antigens in particular, as well as antigens in general."

Dochez and Avery in 1917 had concluded that the soluble specific substance in the blood and urine in pneumonia was probably a protein. Zinsser and his associates were not able to determine definitely whether the residue antigens were heat-stable proteins or nonprotein nitrogenous compounds. It remained for Heidelberger and Avery and others to demonstrate that these materials are polysaccharides.

As a consequence of his studies of bacterial hypersensitiveness and residue antigens, Dr. Zinsser developed hypotheses as to the genesis of several diseases. This was most clearly expressed in his discussion, in 1928, of the allergic hypothesis of rheumatic fever. Wolbach has pointed out that these studies of the effects of residue antigens on sensitized animals "led to generalization of first importance, applicable to responses in bacterial infections in general," and that "this generalization of Hans Zinsser's is perhaps his greatest in the field of immunology. It reflects his training in pathology and his broad understanding of the sequences that compose a disease entity."

At the time when Dr. Zinsser returned to Columbia, in 1913, Noguchi's success in cultivating spirochetes and his observations on reactions produced by injections of luetin opened up a way for the experimental approach to problems of immunization against syphilis. In collaboration with Hopkins and McBurney, Zinsser entered on an extensive investigation of these problems. A series of papers was published during the years 1914 to 1916 on the cultivation of Spirochaeta pallida, on fluctuations in the virulence of these organisms, on spirochetocidal antibodies and on phenomena of immunity to syphilis in animals. Although a practical method for immunization against syphilis was not devised, the work added to the knowledge of spirochetes. It appeared that the culture spirochete had no immunologic relation to the virulent organism. The result established the doubt, still unresolved, as to whether Spirochaeta pallida has been actually cultivated by any one.

As has been pointed out, Dr. Zinsser's experiences in Serbia in 1915 and in Russia in 1923 aroused his interest in typhus fever. In a similar manner his observations of respiratory disease and encephalitis during the World War, 1917 to 1919, moved him to study influenza and virus infections of the central nervous system.

In subsequent years his investigations of influenza were transitory and, as usual, collaborative. They were concerned in part with studies of the influenza bacillus and in part with epidemiology and measures for the control of respiratory disease.

His investigations of herpes and encephalitis were more searching. During the period from 1926 to 1930 he published a series of papers on herpes virus and on the immunologic aspects of the herpes-encephalitis problem, finally, in 1936, bringing together his own observations and the observations of others in an article, "Immunologic Consideration of the Virus Problem."

While Dr. Zinsser was investigating herpes virus he became impressed with the importance of knowing something more about the sizes of virus particles. Finding no precise information on this subject in the literature, he undertook, in collaboration with Tang, to make the necessary determinations, "by measuring viruses against the permeability of graded filters made of collodion by a method more or less analogous to that by which Bechhold attempted to establish a scale of sizes for various substances ranging from crystalloids to Prussian Blue." The results were published in 1927. He estimated that herpes virus, Rous sarcoma virus and bacteriophage "were of a magnitude larger than casein and collargol and smaller than colloidal arsenic." This range of sizes was from 20 to 100 millimicrons. Considering the difficulties and uncertainties, these determinations are amazingly close to the values accepted at present, namely: for bacteriophage, 75 millimicrons for the Rous sarcoma virus, 70 millimicrons for herpes virus, approximately 130 millimicrons. In this original work Zinsser was the first to determine the approximate sizes of virus particles by filtration through membranes of graded porosity.

It was not until 1930, fifteen years after his experiences in the Serbian epidemic, that Dr. Zinsser published his first experimental observations on typhus fever. This paper was obviously preceded by a considerable period of work, in collaboration with Batchelder, on filtration, cultivation and inoculation of the rickettsias of Mexican typhus fever. During the next ten years, some thirty-six papers on typhus were published by Dr. Zinsser in conjunction with one or more of his associates Castaneda, Macchievello, Seastone, Wei, Hagar, Zia, Fitzpatrick, Plotz and Enders. These papers report the results of investigations on nearly all the main problems of typhus fever and related rickettsial diseases. They deal with classification and differentiation of types of these diseases, etiology, trans-

mission, cultivation of Rickettsiae, immunology, epidemiology, prophylaxis and treatment. In the years preceding their publication and in the decade which they cover, other workers in this country, notably Neill, Mooser, Maxcy, Dyer, Rumreich, Badger, Spencer, Parker, Wolbach, Landsteiner, Nigg and others, made numerous original contributions in the same field. Indeed, as Dr. Wolbach has said, the work of Dr. Zinsser and his group and the work of members of the staff of the United States Public Health Service "have been of world wide importance and have patterned the methods of study of rickettsial diseases throughout the world."

Where such extensive investigations of a group of related diseases are being carried on simultaneously by several groups of workers in different parts of the world, it is difficult to separate the contributions of individual members. This is true also of any single group such as Dr. Zinsser's. He, as the constant member of a group of workers, and his associates confirmed and extended the differentiation of the etiologic Rickettsiae by inoculation of animals, by immunologic tests and by cultures in mediums containing fragments of embryonic tissue. Brill's disease, occurring in Boston and New York, was proved to be a variety of European typhus, differing from Mexican typhus, Rocky Mountain spotted fever and the murine rickettsial diseases. Knowledge of the reservoir of typhus virus in rodents was extended by Zinsser and his associates. Their studies of antigenic residues of rickettsias and of bacilli of the proteus group helped to clarify the nature of the Weil-Felix reaction. Finally, special methods were devised by Dr. Zinsser and his collaborators for the cultivation of rickettsias in such large amounts that antigens for use in prophylactic immunization could be produced. These antigens composed of cultures killed with formaldehyde are an enormous improvement on the dangerous preparations of living attenuated rickettsias previously used and promise a means by which populations may be vaccinated against typhus fever. In addition evidence was presented to show that it is possible to obtain a serum with curative properties by the immunization of a horse with rickettsias killed with formaldehyde.

In some remarks made by Dr. Zinsser to me during the summer of 1940 there was an indication that the cultivation of Rickettsia prowazeki of the European variety of typhus may not be entirely feasible by the combination of Cox's egg yolk inoculation with the Zinsser agar tissue culture method or that perhaps the strain in culture may undergo dissociation, affecting its antigenic qualities. Whatever the outcome of subsequent tests may be, it is clear that the work of Zinsser on typhus fever has established broad principles and contributed many practical details of scientific importance and benefit.

Dr. Zinsser's studies of the conditions requisite for the cultivation of the rickettsias of typhus fever, which do not grow except in the presence of living animal cells, led him to investigate the physiologic conditions prevailing in tissue cultures. With Schoenbach, he published one paper on this subject in 1937. He was contemplating further researches on the physiology of cells and the relationships of intracellular parasites to their hosts, and he discussed the experiments he wished he could undertake in new studies of viruses, of leprosy and of cancer.

Of all his investigations, the studies of typhus fever probably gave him the greatest satisfaction. He showed this by his actions rather than by what he said. In talking of the work he rarely referred to the fact that his life had once been in danger from an attack of typhus contracted in the laboratory. As Enders has written: "His gallant and imaginative spirit saw in scientific research high adventure. The investigation of infectious disease became for him a field of battle . . . this lifelong conflict with the agents of syphilis, tuberculosis, typhus, and the rest—satisfied in large part his need for dangerous experience in the pursuit of generous ends."

On March 31, 1939, after a prolonged series of roentgen treatments, Dr. Zinsser wrote: "I'm beginning on long problems as though I had all the time in the world." Although he is no longer in the laboratory, the things he did in his lifetime and the ideas he expressed will furnish material for many "long problems"; his spirit will guide the work.

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Book Reviews

Practical Handbook of the Pathology of the Skin: An Introduction to the Histology, Pathology, Bacteriology and Mycology of the Skin, with Special Reference to Technique. J. M. H. MacLeod, M.A., M.D., F.R.C.P. (London), physician and honorable director of the pathological department of St. John's Hospital for Diseases of the Skin, London; and I. Muende, M.B., B.S., B.Sc. (London), pathologist in charge of outpatients' clinic and lecturer in pathology, St. John's Hospital for Diseases of the Skin, London. Second edition. Cloth. Pp. 415, with 152 illustrations. Price 42 shillings. New York: Paul B. Hoeber, Inc., 1940.

The second edition of MacLeod's "Practical Handbook of the Pathology of the Skin" (the first edition appeared in 1903) has just been published under the joint authorship of MacLeod and Muende. They have adhered closely to the plan of the first edition, as emphasized by the subtitle: "An Introduction to the Histology, Pathology, Bacteriology and Mycology of the Skin with Special Reference to Technique." The book, therefore, still remains unique in the multiplicity of fields in dermatology which the authors attempt to cover and do cover quite successfully even though the book is only 415 pages long. A great deal has been added since the first edition, and much of the obsolete material has been omitted.

It is gratifying to see some of MacLeod's original drawings retained, because they have proved useful to many dermatopathologists in teaching the normal histology of the skin. The colored drawings and, in fact, almost all the illustrations are a most excellent portrayal of the conditions they illustrate. Many of the drawings are so accurate as readily to pass for photomicrographs; yet they have the advantage of emphasizing the cardinal features without emphasizing irrelevant detail.

Just as in the case of the first edition, which was so popular, this book is easy to read. The authors have retained the faculty of being able to say a great deal in simple and concise sentences, without redundancy or repetition.

A sincere review scarcely ever is entirely favorable, even though the book reviewed is admitted to be excellent. The instructions regarding staining are for the most part carried over from the first edition, including descriptions of the technics of many of the old Unna stains, some of which are rarely used at the present time. Conversely, newer staining technic regarding nerve fibrils is not mentioned. For an introduction to technic, the reviewer would suggest that many of the stains might be omitted from consideration or else referred to in footnotes.

The chapter on diseases of the blood and of the skin is open to the criticism of having a brief description of technics for examination of the blood which one would expect any medical student to have learned before beginning dermatologic studies. There is no consideration of newer work in hemocytology by numerous investigators in this country, nor is there mention of monocytic leukemia. The old terms "chloroma" and "leukosarcoma" are retained.

The pathologic descriptions of various dermatoses are clearcut and concise, at times perhaps too much so. The description of precancerous dermatoses seems inadequate, and no mention is made of elastic tissue changes in senile atrophy of the skin.

The authors tend to confuse melanoblasts and chromatophores. They fail to mention dendritic cells. On page 107 they refer to Bowen's disease as an intraepidermal basal cell epithelioma when actually it is a squamous cell carcinoma in situ. No attempt is made to grade epithelial neoplasms, particularly squamous

cell carcinoma. There is a long discussion of the origin of plasma cells which remains almost without change from the first edition.

The authors do not give a clear picture of blastomycosis or distinguish clearly between blastomycosis and allied conditions, such as coccidioidal granuloma and torulosis.

In the discussion of metallic deposits in the skin, no mention is made of the dark field method of the study of argyria or of deposits of hemosiderin in hemochromatosis. There are several other statements or minor points which the reviewer might wish to take exception to. The chapter on dermatophytes and cultural methods is brief but excellent, as is also the chapter on animal parasites of the skin, which latter ordinarily would not be considered germane to a textbook on the pathology of the skin.

Because of the unconventional approach in the method of teaching histopathology and allied subjects concerning the skin, the student may at first find the book a little difficult to use, but a short time spent in studying the arrangement of the contents will make it evident that practically everything can be readily found by use of the index and the list of chapters.

The reviewer would recommend this book to all pathologists interested in histology and pathology of the skin to serve, in the words of the authors, as an introduction and a guide. The book is beautifully written and printed, and the colored illustrations alone are worth the cost of the book. It is needless to say that all dermatologists interested in dermatopathology will welcome this book to their libraries correlating, as it does, the histology, pathology, bacteriology and mycology of the skin with the technics of the various examinations.

Recent Advances in Forensic Medicine. Sydney Smith, M.D., F.R.C.P., D.P.H., and John Glaister, M.D., D.Sc., J.P. Second edition. Cloth. Pp. 264, with 85 illustrations. Price, $4.50. Philadelphia: P. Blakiston's Sons & Co., Inc., 1939.

This handy volume, by Sydney Smith and John Glaister, regius professors of forensic medicine at the universities of Edinburgh and Glasgow, respectively, does not purport to be a textbook of legal medicine. It is a succinct survey of the present status of certain important aspects of forensic medicine. The first 4 of its 13 chapters deal with ballistics and the characteristics of injuries from projectiles. The fully illustrated chapter on the medicolegal examination of hairs contains a contribution on the cuticular scales of hair by Alan Moritz, recently appointed professor of legal medicine at Harvard University; his contribution is based on work done with the authors. A discussion of the precipitin test for blood is followed by a brief consideration of precipitin tests for sperm, bone and muscle. The individuality of the blood as expressed by the blood groups is amply treated, due attention being given to the M and N factors in the determination of paternity. Not only is the interpretation of the results of these various medicolegal examinations evaluated, but the technics of the examinations are given in sufficient detail to make the volume a useful laboratory manual.

The methods for the quantitative estimation of carbon monoxide and alcohol in the blood and for spectroscopic analysis are given. At the present time, in a number of larger cities of the United States, quantitative estimations of alcohol in urine or expired air form the basis of a charge of drunkenness following a motor vehicle accident. The accuracy of deductions as to the amount of alcohol in the blood and tissues, which, after all, is the crux of the matter, based on estimations of the alcohol in the urine or in the expired air, does not seem warranted. At any rate, there are so many variable factors that conclusions must be drawn with care.

The use of ultraviolet and infra-red rays in medicolegal investigation is briefly discussed. A final short chapter on larval infestation and putrefaction has been

contributed by Mearns, of the Institute of Hygiene of the University of Glasgow. In an appendix covering half a page the authors describe Walker's method for the demonstration of powder residues on fabrics.

Hydrocephalus: Its Symptomatology, Pathology, Pathogenesis and Treatment. Otto Marburg, M.D. Cloth. Pp. 217, with 28 illustrations. Price, $3. New York: Oskar Piest, 1940.

The title indicates clearly the scope of the book. The author states in the introduction: "By summarizing and systematizing the facts known today and coordinating them with my own personal experience in this field, I hope to contribute toward the solution of the problem of hydrocephalus." He terminates his work as follows: "That is the problem which has resulted from my investigations on hydrocephalus." The problem to which he refers is that of proving that "hydrocephalus is a disturbance of the water metabolism in the brain tissue." He speaks in many places as though he had established this thesis: "Hence we may plan the treatment to effect a change of the permeability of the blood vessel walls and the barriers, and of water metabolism." When the reader turns to the chapter on treatment to find how this is to be done, he finds that he must depend on roentgenograms and on such products as epiglandol Roche and epiphysan Richter. Yet if the reader goes further he finds that "excessive fetal or infantile hydrocephalus scarcely reacts to x-ray treatment" and "fetal and early childhood hydrocephalus gives the worst results." The author himself states: "It is not possible, therefore, to formulate a general and rational therapy."

The author's rambling remarks in support of this thesis include many doubtful dogmatic statements such as that "the hypophysis causes fluid increase and the epiphysis fluid decrease." The reviewer found his argument entirely unconvincing.

The book would have been improved by more careful proofreading. Oldberg is constantly referred to as Olsberg. Such Germanicisms as the following disturb the reader: ". . . it is ventrally a little inside of the stria terminalis fastened to the upper surface of the optic thalamus." The bibliography, although extensive, is incomplete. The author seems unaware of recent English work on occlusion of the vein of Galen and barely mentions the important Arnold-Chiari malformation.

On the whole, the book succeeds in summarizing in compact and readable form much information about hydrocephalus. If one remembers that the author has merely proposed a personal hypothesis which is far from proved, the book can be useful.

Blood Groups and Blood Transfusions. Alexander S. Wiener, A.B., M.D., Serologist and Bacteriologist in the Office of the Chief Medical Examiner of New York City. Second edition. Pages 306, with 52 illustrations. Price $5. Springfield: Charles C. Thomas, Publisher, 1939.

The changes which are found in this edition are in keeping with the advances in knowledge of blood groups since the first edition appeared in 1935. The number of pages has increased from 220 to 306, the black and white figures from 41 to 52 and the tables from 72 to 84. Two new chapters are added, one on reactions to and complications following blood transfusions, the other on individual identification of stains in forensic cases. Both subjects were treated in the first edition in parts of chapters on other topics. Blood banks and recent studies on the weak agglutinogens A_2 and N_2, on the inheritance of subgroups of the groups A and AB and on individual differences in animal blood are among the added topics dealt with. References have been brought up to date. The favorable comments in the review of the first edition (ARCH. PATH. **19**:609, 1935) hold true, and the book is bound to retain its place as a complete and up-to-date summary of the subject.

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